EVALUATION OF HYPOCHLOROUS ACID (ELECTROLYZED WATER), LACTIC ACID, AND PEROXYACETIC ACID AS SANITIZERS FOR FRESH VEGETABLES

By

JIAMENG WANG

Bachelor of Science in Food Science
Beijing Agricultural University
Beijing, China
2006

Master of Science in Food Science
Oklahoma State University
Stillwater, Oklahoma
2011

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2011

EVALUATION OF HYPOCHLOROUS ACID

(ELECTROLYZED WATER), LACTIC ACID, AND

PEROXYACETIC ACID AS SANITIZERS

FOR FRESH VEGETABLES

Dissertation Approved:

Peter. M. Muriana
Dissertation Adviser

William McGlynn

Deborah VanOverbeke

Dr. Mark E. Payton
Dean of the Graduate College

ACKNOWLEDGMENTS

I am heartily thankful to my advisor, Peter. M. Muriana, whose encouragement, guidance, patience and support from the initial to the final stages of my program have enabled me to develop an understanding of this project and helped with the completion of my studies.

I would also like to thank Dr. McGlynn and Dr. VanOverbeke for being my committee members and for extending their expert advice on my dissertation.

Deepest thanks go to all faculty and staff at the Robert M. Kerr Food & Agricultural Product Center for supporting me in many ways, especially my lab manager, Dr. Kalpana Kushwaha, who helped make my lab work possible and treated me as a family member. Moreover, I would like to thank Dinesh Babu, Preetty Pranatharthiharan, Camilla Bottini, and Hung King Tiong from our lab, who gave me much support during my research.

Last but not least, I want to truly thank my family and friends in United States and China for their encouragement and support.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF COMMON ACRONYMS

Amp = Amperes

ml = milliliters

l = liters

mg/l = milligram per liter

µl = microliter

µg/ml = microgram per milliliter

ppm = parts per million

CFU = colony forming units

cm = centimeters

FAC = free available chlorine

ORP = oxidation reduction potential

min = minute

sec = second

mM = milliMolar

CHAPTER I

INTRODUCTION

The U. S. Centers for Disease Control and Prevention (CDC) has reported that numerous outbreaks were caused by foodborne pathogens associated with fresh produce consumption from 1973 to 1997. These foodborne pathogens caused 16,058 illnesses, 598 hospitalizations, and 8 deaths (Sivapalasingam et al., 2004). The U.S. economic losses due to foodborne pathogens that cause human illness are estimated at 6.5 billion dollars per year (Pimentel et al., 2001). The human economic looses have raised serious concerns about foodborne illnesses linked to foodborne pathogens in food industry.

From harvesting to processing, contamination can occur at any point. This includes the improper use of waste water or manure for production, unhygienic transportation practices, and ineffective sanitation during processing of products meant for consumption. Changes in consumer food preferences and demand for convenience may also be partly responsible for the increase in foodborne illness. An increase in the consumption of fresh fruit and vegetables has been promoted as a more healthy diet. For example, there was a 27% increase in fresh produce consumption in the US from 1970 to 1993 (Roever, 1998). Fresh produce, however, is known to be a vehicle for the transmission of bacterial pathogens capable of causing foodborne illness and many reports refer to fresh produce or minimally processed produce harboring potential foodborne pathogens. *Listeria monocytogenes, Salmonella spp*. and *Escherichia coli* O157:H7

which are frequently linked to foodborne outbreaks, have been isolated from fresh produce during harvesting, processing, and distribution (Nguyen-the and Carlin, 1994).

An outbreak of *Listeria monocytogenes* was reported in 1979 in which involved 23 people became sick (Ho et al., 1986). *Listeria monocytogenes*, a Gram-positive bacterium, is capable of surviving in soil for up to 295 days (Welshimer, 1960) and growing on vegetables subjected to improper distribution and packaging in the food industry (Beuchat, 1996). The symptoms resulting from listeriosis, the disease caused by *Listeria monocytogenes*, are fever, seizures, ataxia, and depressed consciousness. Listeriosis may be acquired during pregnancy and pass to an unborn baby through the placenta (Schuchat et al., 1991).

Another pathogen, *Salmonella*, characterized as a Gram-negative, rod-shaped bacterium, accounts for approximately 1.5 million cases of foodborne illness (Mead et al. 1999). It has been isolated from fresh intact vegetables. For example, 7.5% of the total fresh vegetables samples examined during 1981 to 1983 in Spain were contaminated with *Salmonella spp*. (Ruiz et al., 1987). *Salmonella* food poisoning can infect older adults, pregnant women, infants and people who have compromised immune system problems with multiple bouts of diarrhea accompanied by severe abdominal pain and abdominal cramps (Roever, 1998).

A third pathogen, *Escherichia coli* O157:H7 is another leading cause of foodborne outbreaks associated with fresh produce. An estimated 73,480 foodborne illnesses due to *E. coli* O157:H7 infections occur each year in the U.S. (Mead et al., 1999). In the U.S., between 1982 and 1994, 6% of outbreaks associated with *E. coli* O157:H7 resulted from the consumption of vegetables (Doyle et al., 1997). The clinical features of *E. coli* O157:H7 infections begin with abdominal cramps, bloody diarrhea and a low grade fever. The disease can cause 3 to 5% of people who develop haemolytic uraemic syndrome (HUS) to die (Mead and Griffin, 1998).

By investigating how pathogens can be reduced during post-harvest processing of produce, we can learn how to improve food quality. In 1999, in response to the requirement of produce safety issues, the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) have commenced establishing guidance on good agricultural practices (GAPs). The document entitled, "Guidance for Industry – Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" addresses the issue of microbial food safety and good agricultural and management practices on most minimally processed vegetables and fruits. The document addresses processes during producing, harvesting, transporting, washing, sorting, packing, and retailing to consumers. FDA has analyzed and evaluated several preventive control measures for the reduction or elimination of microbial hazards on fresh and fresh-cut produces.

Washing is considered to be a substantial and direct method to remove pathogens from food surfaces. This step involves the application of water and processing chemicals under various conditions to the produce surfaces. Some pathogens transferred from vegetable surfaces into the wash water may resist the action of the antimicrobials, resulting in some bacteria being left on vegetable tissues. These pathogens may also have naturally physical barriers to prevent them from contacting the antimicrobial agents directly. Based on the features of microorganisms and different characteristics of chemicals (i.e., unacceptable sensory impact on produce and unavoidable residue after treatment), antimicrobial chemicals allowed for use in food processing should be safe, convenient, and effective.

The use of sanitizing antimicrobials for fresh vegetables varies depending on the pH of the disinfectants, contact time, types of vegetables, and the pathogen targeted. Three antimicrobials commonly used on washing fresh produce are organic acids (lactic acid), peroxyacetic acid, and sodium hypochlorite (Electrolyzed water). Organic acid, especially lactic acid, is successfully used as a disinfectant applied to produce surfaces for the purpose of reducing foodborne pathogens (Koseki et al., 2004). The efficacy of peroxyacetic acid which has been

introduced to reduce microbial loads on fresh-cut vegetables is often used as well (Hilgren and Salverda, 2006). Several studies have shown that sodium hypochlorite is a safe and effective antimicrobial agent for vegetables, and the only chemical agent currently allowed by federal regulations for fresh vegetables and fruits with a maximum concentration of 200 ppm (Venkitanarayanan et al., 1999; Kim et al., 2001; Huang et al., 2008). All of the above ingredients have been shown to be effective antimicrobials and used individually or in combination for microbial reduction of pathogenic bacteria in foods (Zhang and Farber, 1996; Hua and Reckhow, 2007). Of these three antimicrobials, electrolyzed water is the easiest to generate with current automated technology.

Sodium hypochlorite is listed as a safe and suitable ingredient for maintaining processing water quality by FDA. It been introduced and demonstrated to minimize the risk of infection associated with the consumption of fresh vegetables or to eliminate contamination from the environment. Electrolyzed water is produced by electrolysis of a weak brine solution of sodium hypochlorite through an electrolytic cartridge. During the generation of electrolyzed water, the pH is usually maintained between 6 to 6.5, which maintains the equilibrium for hypochlorous acid (HOCl), the active antimicrobial component of electrolyzed water instead of hypochlorite that would be the active agent at neutral pH or higher (Hrivoca et al., 2008). Its concentration is regarded as the amount of free available chlorine, in the form of HOCl, present in the water that can come into contact with microbial cells. Electrolyzed water has been studied to prove that it is an effective, relatively inexpensive, and environmentally friendly new technology for potential use as a disinfectant on food produce over other traditional cleaning agents (Koseki et al., 2002; Deza et al., 2003).

Electrolyzed water has been used as a sanitizer on produce to reduce pathogens. It is an oxidizing agent and has been reported to possess strong bactericidal activity against a variety of pathogenic bacteria including *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* (Park et al.,

2004; Kim et al., 2007; Huang et al., 2008; Venkitanarayanan et al., 1999). In recent years, electrolyzed water has been shown as an effective antimicrobial agent for reducing pathogens on fresh vegetables, such as lettuce (Delaquis et al., 1999; Izumi, 1999; Park et al., 2001; Koseki et al., 2001; Koseki et al., 2004a; Zhang and Farber, 1996), tomatoes (Bari et al., 2003; Deza et al., 2003; Hyun et al., 2005), carrots (Izumi, 1999; Singh et al., 2002), spinach (Guentzel et al., 2008; Park et al., 2008), peppers (Izumi, 1999) and fruits (Koseki et al., 2004b; Rico et al., 2007). In other research, electrolyzed water has been studied on egg shell sanitation (Russell, 2003), poultry surfaces (Park et al., 2002), and on seafood (Huang et al., 2006). One of the difficulties in comparing studies with hypochlorous acid is that it is an oxidant which can interact with organic matter and the relative volume of sanitizer to organic matter treated will affect efficacy. However, these studies could be improved by using a standardized surface area being tested or sanitized, and give a better idea of efficacy on various products.

These studies may also be improved by using different antimicrobials in combination with electrolyzed water. Antimicrobials such as lactic acid and peroxyacetic acid are also used as sanitizers for food produce. Lactic acid, a naturally occurring organic acid, is commonly used as food preservative, decontaminating and flavorant agent due to its absence of acute and chronic toxicity (Oh and Marshall, 1993). The bactericidal effects of lactic acid have been studied on poultry and meat (Woolthuis and Smulders, 1985; Zeitoun and Debevere, 1990). Along with an effective action at low temperatures, peroxyacetic acid has used as a sanitizer on fruits (Wisniewsky et al., 2000; Farrel et al., 1998). These acids may be used in combination with electrolyzed water to reduce pathogens.

The use of antimicrobials to control microorganisms in food products has raised public concerns about food safety. These concerns have prompted the use of combinations of antimicrobial agents for produce (Oh and Marshall, 1993). Electrolyzed water is more effective, less dangerous, and less expensive than other antimicrobials. The greatest advantage of

electrolyzed water relies on its less adverse impact on the environment and on workers' health. The objective of this study was to examine the effectiveness of electrolytically generated hypochlorous acid, alone and in combination with lactic acid or peroxyacetic acid, and in maintaining a standardized surface treatment area. The effectiveness of sodium thiocyanate spray followed by immersion in electrolyzed water will also be conducted to compare with electrolyzed water treatment alone.

CHAPTER II

REVIEW OF LITERATURE

History of food microorganisms

The precise time humans realized there were microorganisms in food can be traced back to approximately 8,000 years ago. During this period, the method of food preservation can be divided into food gathering and food producing. It is presumed that the problems of food spoilage and food poisoning appeared at that time also (Jay, 2000). Jews were the first to use salt in the preservation of different kinds of food. Later, the Chinese, Greeks, and Romans began to use the same method on fish to extend shelf life. Butter, wheat, and barley were introduced by culturing, also known as fermentation today. Beer and cheese, both fermentation products, have been traced as far back as 7000 BC. The processes of fermentation involved the transformation of simple raw materials into value-added products by utilizing different naturally occurring microorganisms (Farnworth, 2005).

People believed that there was a connection with food spoilage and illness. Although there were still no direct and specific evidence to determine the relationship between microorganisms and food spoilage, people initiated different methods to remove undesirable organisms from food in order to extend the shelf life. When people began to notice the importance between food spoilage and food preservation, many advances in food technology were introduced and implemented in food systems. In 1810, the first major technique for food

preservation by canning was patented. While Pasteur perfected his pasteurization methods of using heat to destroy microorganisms, he also informed the public of his discovery in 1860, later this process became known as pasteurization (Jay, 2000).

In 1842, with a new concept of preservation by immersing food in ice, people found an easy way to preserve fish and some meats on a commercial scale. For fruits and vegetables, Franks designed a patent for food under the atmosphere of CO_2 in order to extend the shelf life. The first commercial use of controlled atmosphere storage was used on apples in 1928 (Jay, 2000). In 1992, another commercial facility was designed to preserve food using irradiation. All of these innovative preservation applications were implemented to reduce undesired food microorganisms that result in food spoilage. However, the true identification of microorganisms and how they contaminate food were uncovered in conjunction with the development of methods to preserve them.

As early as 1658, the occurrence of microorganisms had just begun to be understood, so the connection between microorganisms and diseases acquired from food was less well understood. A. Kircher was the first person to investigate the appearance of microorganisms in spoiling foods. Francesco Selmi was the first person to presume the interaction between illnesses and certain foods. In 1888, the association between foodborne diseases and microorganisms was established by Gaernter, who had successfully isolated *Salmonella enteritidis* from meat. This discovery excited other researchers to continue such studies by isolating disease causing agents from food products (Jay, 2000). Scientists soon discovered *Clostridium botulinum, Bacillus cereus* and *Yersinia enterocolitica* were associated with foodborne diseases. As the field of food microbiology developed and matured, the involvement of pathogenic bacteria which includes *Escherichia coli, Listeria monocytogenes, Salmonella spp., Shigella, clostridium botulinum, Bacillun cereus and Staphylococcus,* and others have become better understood. Studies to reduce

illnesses associated with foods have helped to define the involvement of foodborne pathogens and spoilage organisms as well as methods to control them.

Microorganisms of importance to the food industry

The U. S. CDC has estimated that 76 million foodborne illnesses occur each year in the U.S. These illnesses cause 325,000 hospitalizations, 5,000 deaths, and $ 6.9 billion financial compensations on annual medical costs and productivity losses (Mead et al., 1999; Crutchfield and Roberts, 2000). According to a total of 190 produce-associated outbreaks in the USA from 1973 to 1997, pathogenic bacteria were responsible for causing the rise in foodbrone illnesses from 0.7% in the 1970s to 6% in the 1990s.

The common foodborne pathogens are made up of all pathogenic strains of *Listeria monocytogenes, E. coli*, *Salmonella spp., Staphylococcus aureus and Campylobacter*. Among these bacteria, *Campylobacter* infections are one of the most common causes of diarrheal illness in the U.S, contributing to an estimated 2.4 million infections annually. *Salmonella* ranks second to *Campylobacter* for number of cases of foodborne illness. During 2005 and 2006, there were four multistate outbreaks of Salmonellosis that found 450 people sickened in 21 states after consuming contaminated tomatoes in restaurants (CDC, 2007). *Salmonella* is becoming the second most numerous foodborne illness pathogens. From June to July 2007, it has been reported that, there were 65 and 425 known illness from *Salmonella* in the U.S. by eating vegetables and peanut butter, respectively. There were 450 people in 21 states sickened by the consumption of tomatoes contaminated with *Salmonella* in 2005. *E. coli* O157:H7 was also reported as foodborne bacteria to contaminate food. In 2006, at least 183 people in 26 states were affected by eating bagged spinach and lettuce in fast-food restaurants infected by *E. coli* O157:H7 (Grant et al.,

2006), and183 people were reported to be affected by consumption of bagged spinach contaminated with *E. coli* O157:H7 in the same year (Pangloli et al., 2009). Human infection associated with the consumption of raw cabbage containing *Listeria monocytogenes* was reported in Canada in 1981 (Schlech et al., 1983), and many researchers had investigated that *Listeria monocytogenes* could be found associated with decaying vegetation and soil (Welshimer, 1968). These outbreaks cost the United States food industry and government an estimated $2.9 to $ 6.7 billion annually (Powell and Attwell, 2000).

The role of microorganisms' growth on produce

The ability of bacterial pathogens to contaminate produce, multiply, and incite disease is not necessarily a result of their ability to produce enzymes, toxins or other virulence factors to harass the structure or function of plant cells. However, some foodborne bacteria are able to possess such mechanisms (Liao et al., 2003). In natural food systems, microorganisms incidentally adhere to conditional surfaces providing sufficient nutrients for growth and survive. Based on the typical characteristics of microorganisms (small size, ease of dispersal, tolerance of extreme condition, and physiologic diversity), once the microorganisms become attached surfaces, they form a diverse bacterial community that will produce polymers (EPS) to enhance the establishment of layers of cells on solid surfaces (McMeekin, et al., 1997; Chavant et al., 2002). Francis et al. (1999) mentioned that the development of foodborne bacteria on ready-to-eat (RTE) vegetables relies on the properties of microorganisms and vegetables, and a series of processing steps before serving to market. Foodborne bacteria are prone to display their ability to multiply and grow on food surfaces, especially when the normal cell organization of food surfaces has been destroyed.

Biofilms are often thought of only being found on inert surfaces such as equipment, but multilayers of bacterial cells attached to the surfaces of plants will gather organic and inorganic debris and nutrients to form a microbial biofilm on plant surfaces (Kumar and Anand, 1998). Different types of bacterial biofilms have various abilities to protect the bacterial cells from environmental stresses, and provide different mechanisms to support microbes to adapt to those stresses (Monier & Lindow, 2005). The process of adhesion is complicated and complex, because it based on many factors from both the surface and bacterium cell side (Beczner and Vidács, 2009).

Several environmental factors can influence the potential growth of microorganisms on food produce surfaces, such as the temperature, soil, moisture content, water activity, pH, and the availability of organic matter. Temperature is considered to be the most influential parameter to affect the presence of microorganisms presented in the soil. When nutrients are limiting, bacterial survival increases as the temperature decreases. Under certain environmental conditions, strains of *Listeria monocytogenes, E. coli*, and *Salmonella* may survive from 6 months to several years, depending on conditions (Nicholson, 2005). Soil is another important factor in determining the loads of bacteria on plant surfaces. The concentration and survival of microbes also depends on the types of soil being used. The more acidic the soil, the more microbes tend to grow (Beczner and Vidács, 2009). Water activity also has an impact on the growth of bacterial microorganisms. When confronting low water activity, microorganisms are able to regulate their internal environment by accumulating compatible solutes to adjust themselves to surrounding conditions (McMeekin, et al., 1997). Brown et al. (1997) found five strains of *E. coli* that showed a high acid tolerance that correlated to the fatty acid composition of their cell membrane when exposed to a lethal acid challenge (pH=3; Brown et al., 1997). This is similar for *Salmonella*, which has been demonstrated to grow on sliced tomatoes at pH of 3.99 (Wei et al., 1995). However, a pH of 4.5

or higher in produces has been generally recognized as a pH range allowing growth of bacteria (Roever, 1998).

Fresh produce can be served as a source of foodborne bacteria, because of their location (outside) and potential visitation by animals that harbored and shed microbial pathogens. The general composition of fresh vegetables is 88% water, 8.6% carbohydrates, 1.9% proteins, 0.3% fat and 0.84% ash. These conditions have sufficient nutrients to provide a suitable living place for bacterial pathogens to grow (Jay, 2000). Since bacteria populations establish themselves on growing vegetables, and if there is no lethal treatment to remove them before serving to market, this problem can be amplified by potential growth prior to consumption (Roever, 1998). In addition, many studies have suggested that vegetable contamination could occur both at the pre-harvest and post-harvest phase (Berger et al., 2010).

Either the unique characteristics of bacterial pathogens or the surrounding environmental factors affect the tendency of vegetables being a vehicle for transmission of foodborne disease. Ensuring the security of current and future food supplies becomes the issue and challenge facing consumers all over the world.

Approaches to improve food safety

Government regulations

The Food and Drugs Administration (FDA) has listed the sources of pathogenic microorganisms for concern on fresh produce and conditions that may influence their survival and growth. For the pre-harvest phase these are: soil, irrigation water, green or inadequately

composted manure, air (dust), wild and domestic animals, human handling, and water intended for other uses (pesticides). For the post-harvest phase, the potential sources of foodborne pathogens include: human handling, harvesting equipment, transport containers, wild and domestic animals, air (dust), wash and rinse water, sorting, packing, cutting, further-processing equipment, ice, transport vehicles, improper storage, improper packaging, cross contamination, improper display temperature, improper handling after wholesale or retail purchase, and cooling water. Contamination could occur at any point under improper handling and storage prior to consumption. Therefore, control measures in the food handling chain should be able to prevent contamination of fresh produce with microbial pathogens, physical contaminants, or dangerous levels of chemical residues to assure that these foods are wholesome and safe for human consumption. The methods include Good Manufacturing Practices (GMPs), Good Agricultural Practices (GAPs), and Hazard Analysis Critical Control Points (HACCP).

Good Agricultural Practices are established by the FDA as guidelines to reduce or eliminate pathogen contamination in the field or in packinghouse operations. Because GAP guidelines need additional information on examining the microbiological hazards once the initial evaluation has been performed, the costs associated with implementing a GAP food safety program vary considerably due to numbers of factors, such as the ability of growers to develop food safety program, the ability to maintain necessary documentation, and the number of water sources used for irrigation. Based on the fact that compliance with GAPs is simply a guideline and not mandatory, the FDA conducts GMPs as rules to be implemented by processors in the production environment. Similar to the way GMPs and SSOPs (Sanitation Standard Operating Procedures) support meat HACCP. Good Manufacturing Practices accompanied with GAPs play a role as prerequisite programs to be the foundation for establishing HACCP systems for produce and vegetable manufacture and processing.

Since contamination can occur during produce growing, harvesting, washing, sorting, packing, and transport, producers should be aware of any details about safety problems in food manufacturing. Water used in production involves many different field operations including irrigation, application of pesticides and fertilizers, cooling, and frost control. Therefore, fresh produce can be contacted by water directly or indirectly, so the quality of water dictates the potential for pathogen contamination. Water, both agricultural water (ground water) and processing water, is considered to be a carrier of many pathogenic bacteria.

Groundwater, surface water and human waste water are commonly used for irrigation in the pre-harvest period. The risk of disease transmission from pathogenic microorganisms present in irrigation water has the potential for contamination. Surface water and human waste water is usually of very poor microbial quality and requires pre-treatment before applying onto vegetables as irrigation water, but very few of these receive such treatment. Groundwater also has the possibility to be contaminated with microorganisms present in surface runoff (Steel and Odumeru, 2004).

Water is also used during the post-harvest handling of fruits and vegetables, although water is considered to be a useful tool for reducing potential contamination, it can still provide an opportunity for dispersion of bacterial pathogens. In order to eliminate bacterial pathogens associated with fresh produce from processing water, the Food Quality Protection Act (FQPA) and the Antimicrobial Regulation Technical Corrections Act (ARTCA) have regulated the use of additives in water to inhibit microorganisms since 1996. The Food Quality Protection Act has changed the definition of “food additive”, which had a significant impact on the regulatory authority for products that are used in food contact application. Antimicrobial chemicals are certificated to be useful in eliminating microbial pathogens in water, reducing microbial loads on the surface of produce and minimizing the potential hazard for food consumption.

Food industries have been using antimicrobials to reduce pathogens on food produce over 100 years. Antimicrobials can be classified as “traditional” and “naturally occurring” (Davidson and Harrison, 2002).

A number of traditional antimicrobials are allowed to be applied to foods by most international regulatory agencies, such as acetic acid. However, only a few naturally occurring antimicrobials are approved for use in foods, such as nisin (Davidson and Harrison, 2002).

The goal of using food antimicrobials in food is to inhibit spoilage microorganisms in order to preserve food quality and prolong shelf life. Several exclusive antimicrobials are used to control and reduce the growth of specific foodborne pathogens. For example, organic acid has been used as a spray on beef carcass surface to reduce *E. coli* O157:H7 and lysozyme has been used to inhibit population of *C. botulinum* in pasteurized process cheese (FDA, 2000).

A population of pathogenic bacteria could be killed when exposed to a sufficiently high concentration of antimicrobial compounds; however, some bacterial pathogens still survive because they possess a degree of natural resistance or undergo mutation or genetic exchange (Bower and Daeschel, 1999). The natural resistance responses of microorganisms to antimicrobials or sanitizers are described as innate, apparent, or acquired. The mechanisms of innate resistance are those naturally associated with a microorganism and may be due to different types of cellular barriers preventing entry of the antimicrobial, insufficient biochemical targets for antimicrobial attachment or microbial inactivation, or lacking of inactivation of antimicrobials by microbial enzymes (Bower and Daeschel, 1999). The mechanisms of apparent resistance are dependent upon the conditions of application, and may result be affected by the food composition, or food pH value and polarity (Davidson, 2001). The mechanisms of acquired resistance, which are obtained by genetic changes, may occur through mutation or acquisition of

genetic material from plasmids (Russell, 1991). Nevertheless, all antimicrobial chemicals adding in processing water should be in accordance with FDA regulations.

Ingredients allowed for use on fresh produce

Due to the fact that bacteria of public health concerns can survive for extended periods on fresh produce, and under favorable conditions specific fresh-cut vegetables even may provide a suitable condition for growth of pathogenic bacteria, an important step in the processing of fresh vegetables is thorough washing. Washing can help to remove compounds, which may support the growth of microorganisms released during slicing and shredding (Bolin et al., 1977). Washing has been considered as an indispensible treatment for reducing a portion of the pathogens which intend maybe spread, from the surface of produce during processing (Nguyen-the and Carlin, 1994). However, using only water cannot assure the compete removal of pathogens from food surfaces. Washing treatment is often accompanied with antimicrobial chemicals to reduce microbiological loads. Some antimicrobial chemicals allowed on produce, fruits, and vegetables include chlorine dioxide (ClO_2), lactic acid, ozone, hydrogen peroxide, peroxyacetic acid (PAA), and chlorine.

Aqueous chlorine dioxide ($ClO_{2)}$ has been used in fresh produce to determine its effectiveness on killing foodborne pathogens. Singh et al. (2002) conducted aqueous ClO_2 treatment of shredded lettuce inoculated with 1 ml three-strain cocktail of *E. coli* O157:H7 (C7927, EDL933, and 204P) and reported a 1.72 log reduction ($P < 0.05$) in comparison with control (sterile water wash) when 20 mg/l of aqueous ClO_2 was used for 15 min. Though the reduction in inoculated strains was significant with respect to the control, the implementation of

applicator to generate gaseous ClO_2 for treatment is relatively expensive and complicated (Wu and Kim, 2007).

Alternatively, Ozone has been used as another chemical for foods. Singh et al. (2002) tested the ozonated water (5.2-, 9.7-, and 16.5 mg/l) dipping treatment on lettuce and baby carrots. The results observed in this study have shown a significant ($P < 0.05$) reduction on baby carrots after dipping in 5.2 mg/l ozonated water for 10 min. However, there was no significant difference on reducing microbial populations on lettuce. Similarly, Ölmez (2009) did dipping treatment (2 min) with ozonated water (1.5 ppm) on lettuce inoculated with *E. coli* (ATCC 25922) and obtained 1 log CFU/g reduction.

Oh and Marshall (1994) tested the efficacy of monolaurin combined with lactic acid against *L. monocytogenes* (10^5 CFU/ml) on precooked crawfish tail meat. The results showed inhibition of the inoculated bacterium with increasing concentrations of the lactic acid (56, 112, and 224 mM) at 0.72 mM monolaurin with bacteria being below detectable levels after 10 days at the highest lactic acid concentration. However, the use of lactic acid on reduction of bacterial load in vegetables has not been reported.

Peroxyacetic acid (PAA) has been approved for use on food processing equipment as a sanitizer by the U.S. FDA as well as application on leafy vegetables. Zhang et al. (2009) conducted a study using lettuce leaves by inoculating 100 µl of a five strain mixture of *E. coli* O157:H7 and sipping for 2 h in 800 ml PAA solution at 10, 20, and 30 ppm for 1.5 min with agitation. A reduction of 2.97 log CFU/ml was shown at 30 ppm PAA compared to the initial *E. coli* O157:H7 population of 5.6 log CFU per piece. In another study, Wright et al. (2000) reported a 2 log reduction of *E. coli* O157:H7 by using 80 ppm PAA as treatment, but the interval between inoculation and treatment was only 30 min. Researchers also obtained comparable results at this

concentration by using apples inoculated with a non-pathogenic *E. coli* but had to increase the concentration to 1000 ppm PAA to get the same units of reduction (Sapers, 2001).

Chlorine has been used for many years to reduce pathogens as a disinfectant in wash, spray, and flume water. It is usually applied at a concentration of 50 to 200 ppm with a contact time of 1 to 2 min (Liao et al., 2003). Zhang and Farber (1996) reported a 1.3-1.7 and 0.9-1.2 log CFU/g reduction of *L. monocytogenes* on fresh-cut lettuce and cabbage, respectively after chlorine (200 ppm) treatments. Chlorine rinse is a common processing method for pathogen reduction, yet various other treatments have been introduced as alternatives for eliminating or substantially decreasing bacterial populations followed by chlorine, such as hydrogen peroxide (Lillard and Thomson, 1983), gamma irradiation (Katta et al., 1991), and chilling (Vivien et al., 2000).

Electrolyzed water used in the food industry

The Food and Drugs Administration (FDA) has recommends that processors should implement processing interventions during processing to reduce potential hazards and cross-contamination.

Electrolyzed water, or sodium hypochlorite solution, are effective antimicrobial agents that FDA have allowed for use on fresh produce to eliminate pathogens. Some HACCP programs have emphasized the addition of chlorine in washing water to avoid accumulation of bacteria and cross-contamination on plant cells (Wilcox et al., 1994). Depending on the conditions, a 50 to 200 ppm chlorine solution is widely used as a sanitizing agent in the food industry. Chlorine is accepted due to its antimicrobial, but nontoxic effects on produce (Smith, 1962).

Production of electrolyzed water

Electrolyzed 'hypochlorous acid' solution (EW) constitutes a modernized automated generation system that has recently been resurfaced for use as an antimicrobial oxidizing agent. There are many different types of electrolysis apparatus used to produce EW, the two compartment batch-scale electrolysis apparatus is commonly and generally used to produce EW in laboratories (Mahmoud et al., 2006). Japan is one for the biggest manufacturers of electrolyzed water machines. Electrolyzed water has been promoted as a highly promising water treatment solution for several decades in Japan.

Electrolyzed water uses electrolysis in order to dissolve sodium chloride (NaCl) in deionized water, it will immediately dissociate into negatively charged chlorine (Cl^-), hydroxyl (OH^-) and positively charged sodium (Na^+), hydrogen (H^+). The chlorine and hydroxyl ions are moved and concentrated at the vicinity of the anode, while each ion releasing an electron (e^-) to become oxygen molecules, chlorine molecules and hypochlorous acid (HOCl) surround the anode compartment. In the meanwhile, the positively charged sodium ion receives an electron, and then combines with water molecules, forming sodium hydroxide and hydrogen molecules. In some systems, a compartments ion-exchangeable membrane separates the electrodes into anode and cathode, respectively (Mahmoud et al., 2004; Mahmoud et al., 2006). Effluent streams situated near the anode or cathode carry off the different chemical constituents attracted to these polar electrodes.

Acidic electrolyzed water (AEW) generated from the anode side (represented by a low pH, high ORP, and the presence of hypochlorous acid) has been used as an antimicrobial to

reduce foodborne pathogens attached to the most surface of food products, such as lettuce, cabbage, tomatoes, and spinach. Neutral electrolyzed water (NEW) is generated like AEW but a part of the product accumulated near the anode and redirected into cathode chamber by increasing the content of ClO^- ions. Although the bactericidal effect of NEW is not as strong as AEW, NEW is still considered as a sanitizer in the food industry due to being less corrosive to processing equipment or workers' hands (Deza et al., 2003). Alkaline electrolyzed water (AK-EW) generated from the cathode side, has also proved to be an effective disinfectant for food contact surfaces (Pangloli, et al., 2009).

The mechanism of electrolyzed water

According to the conclusion of Park (2002), the mechanism of inactivation of microbial cells by EW is not clear, but is contributed to the oxidative action of hypochlorous acid (HOCl) which is affected by free available chlorine (FAC), low pH and high oxidation reduction potential (ORP). Hypochlorous acid (HOCl) can inactivate bacterial cells by inactivation of enzymes which participate in metabolism (Hurst et al., 1991), inhibition of ATP generation (Barrette et al., 1989), retardation of active transport (Hurst et al., 1991) and oxidation of cell surface sulfhydryl compounds (Leyer and Johnson, 1997; Park et al., 2002).

Because of the sensitivity of most outer membranes of bacterial cells, hypochlorous acid (HOCl) at low pH can efficiently puncture cells membranes and inactivate them (Venkitanarayanan et al., 1999). The high ORP of a treatment solution also might be another factor affecting microbial inaction (Kim et al., 2000a). The high ORP, due to the oxygen released

by the rupture of the unstable bond between chloric radicals and hydroxyl in electrolysis, provides frequent changes in the electron flow in the cell to modify metabolic fluxes and promote ATP release. Kim et al. (2000b) suggested that the ORP of electrolyzed water for bacteria inactivation is a primary factor to consider for inactivation of microorganisms.

The flow rate has been investigated for its ability to affect the concentration of EW. With increasing electrolytes, a high flow rate will decrease the ORP and free available chlorine of EW due to the less residence time in the electrolytic cell (Ezeike and Hung, 2004).

Use of electrolyzed water on foods

Use of electrolyzed water on fresh vegetables

Fresh vegetables, especially ready-to-eat (RTE) vegetables, have been found to harbor large and diverse populations of indigenous bacteria after minimal processing (Nguyen-the and Carlin, 1994), some of which may be pathogenic microorganisms. Roever (1998) demonstrated four factors as the explanation of the interaction between bacterial microorganisms and fresh vegetables: the growing characteristics and survival capabilities of different microorganisms, the physiological state of the plant tissue, the surrounding growing environment, and the effect of food processes and practices on microbial populations.

Vegetables need to receive some degree of processing before being placed in commercial distribution. The increasing presence of cut surfaces or damage plant tissues on vegetables provide a nutrient condition for microbial growth, especially when lacking sufficient processing to ensure sterility or even microbiological stability (Nguyen-the and Carlin, 1994). Fresh produce

may harbor a diverse group of microorganisms which includes the potential hazard of being contaminated after harvesting, and contamination will extend to occur during transportation (Splittstoesser, 1970). Most vegetables have a pH of 4.5 or higher value which can allow the growth of a variety of bacteria (Roever, 1998).

Roever (1998) mentioned that some plant tissues have naturally occurring antimicrobials to protect themselves against the growth of pathogens. The two principle determinants to prevent growth of pathogens on fresh produce are pH and storage temperature. When the pH value of sliced or cut tomatoes as low as 3.99, many studies have reported that *Salmonella* can still grow at this pH (Asplund and Nurmi, 1991; Wei et al., 1995). The survival of microorganisms on fresh produce is also determined by temperature, because strains of *L. monocytogenes*, *Y. enterocolitica*, and *Aeromonas hydrophia* could be present and possibly grow on fresh vegetables even during refrigeration storage temperatures (Nguyen-the and Carlin, 1994).

Fresh and fresh-cut vegetables

Maria et al. (1996) demonstrated that lactic acid bacteria (LAB) showed the highest growth rate (μ_{max} as log $CFUg^{-1}day^{-1}$) on carrots due to the sugar content of this product. Abadias et al. (2008) compared the efficacy of standard sodium hypochlorite treatment (SH) (120 ppm) and neutral electrolyzed water (NEW; 50 ppm) on shredded carrots, and other vegetables (40 g) for dipping treatment at 3 min followed by rinsed with deionized water for 1 min. The reduction of native bacteria showed a significant difference ($P < 0.05$) on carrot comparing to other vegetables, and also demonstrated that the bactericidal activity of NEW (50 ppm) against natural bacteria associated with fresh vegetables was as the same effective as chlorinated water (120

ppm) for the same treatment time. In Izumi's (1999) study of rinsing with EW at 20 ppm FAC for 4 min, EW reduced the microbial loads 0.4 to 0.7 log CFU/g on the surface of carrot slices.

Lin et al. (2005) conducted serial experiments to determine the efficacy of strong electrolyzed acidic oxidizing water (AC water) and alkaline electrolyzed water (AK water) with 50 ppm FAC on bell pepper samples by dipping method. The reduction of aerobic plate count (APC) obtained was around 1.0 log CFU/g on pepper after dipping in AC water at 15 min and AK water at 5 min. The author also suggested using AC water followed by AK water to wash vegetables, which may reduce the undesirable odor from AC water left.

Tomato can be easily grown from seeds, but may be contaminated with pathogenic bacteria (i.e., *Salmonella*) through contact with animal excreta, contaminated soil, infected water, and improperly composted manures as fertilizers during harvesting. To control cross-contamination by pathogenic bacteria, tomatoes can be washed using chlorinated water before shipping. Hyun et al. (2005) conducted a study to evaluate the effectiveness of chlorinated water (HOCl = 200 ppm) as well as PAA at 87 ppm to reduce *Salmonella* population on smooth surfaces and stem scar tissue of green tomatoes. Unwaxed green tomatoes were inoculated at 10 sites per fruit with 10 µl per site. One set of tomatoes was evaluated immediately after washing treatment; the other set would be evaluated after stored at 20^{o}C with 95% relative humidity (RH) for 5 days. Individual HOCl (200 ppm) treatment showed a significant reduction in *Salmonella* on tomatoes, approximately up to 5.0-log reduction on smooth surface inoculation after 60 and 120 s with respect to untreated tomatoes (1.65- and 2.53 log) and stem scar (1.18- and 1.27 log), respectively. The populations of *Salmonella* were undetectable (1.0 x 1.0^{2} CFU/ml) after 5 days of storage.

Zhuang et al. (1995) used chlorine solutions at 0, 50, 100, 200, and 300 (µg/ml) to determine the inactivation of *S. Montevideo* on mature green tomato surfaces and in stem core

tissues. Batches of six tomatoes (25^{o}C) inoculated with *S. Montevideo* were immersed in 1-l of chlorine solution followed by agitation for 2 min. After removal from the solutions, they were separately analyzed for surface and core tissues populations of *S. Montevideo*. The results revealed that a significant ($P < 0.05$) reduction in the surface populations was observed when dipping in solutions containing 60 and 110 ppm, respectively. With concentration of solution increased (320 ppm), there was no significant reduction achieved. On the basis of their study, the author suggested to use a free chlorine solution concentration approximately 200 ppm on rinsing tomatoes in packinghouses.

Green leafy vegetables

Fresh-cut vegetables provide a high level of moisture, nutrients, and surface area for helping microbial growth compared to the original intact product. Green leafy vegetables have a potential risk for microbial contamination due to its inherent contamination and its exposure to unpredictable factors during harvest and processing.

In the Park et al. (2008) study, spinach leaves were inoculated with 10^{6}-10^{7} CFU/g of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890) before being immersing 500 ml of deionized water (DI), acidic electrolyzed water (AC-EW), alkaline electrolyzed water (AK-EW), or AK-EW + AEW for 15 and 30 s, and 1, 3, and 5 min, respectively. Acidic electrolyzed water at 37.5 ± 2.5 ppm FAC showed a significant difference ($P < 0.05$) than AK-EW or control treatment on spinach. They observed 1.97-, 2.95-, and 3.24-log CFU/g reduction of *E. coli* O157:H7 resulting after 15 sec, 30 sec, and 1 min treatments, respectively. After 3 min, the reduction of *E. coli* O157:H7 was more than 3.50 log CFU/g.

Koseki et al. (2004) conducted a study to examine the effect of alkaline and acidic electrolyzed water (AK-EW and AEW) on intact lettuce leaves, which inoculated with mixed strains of *E. coli* O157:H7 and *Salmonella*. The 10 pieces of lettuce leaf (5 cm x 5 cm) were dipped in 1.5-l of AK-EW, distilled water (DW), or AEW (40 ppm of FAC) solution, respectively. After agitating vigorously at 150 rpm for 5 min, the lettuce samples were followed by immersion in 1.5-l of AEW for 5 min. The final step was to rinse the treated lettuce with 1-l deionized water twice. The best reduction was achieved when lettuce was treated with AK-EW and subsequently dipped in AEW, with showing that the reduction of *E. coli* O157:H7 and *Salmonella* on lettuce was approximately 1.8 and 1.7 log CFU/g, respectively.

Abadias et al. (2008) also examined the same study on lettuce by using neutral electrolyzed water (NEW) to reduce the populations of *E. coli* O157:H7 and *Salmonella*. Neutral electrolyzed water at 89 ppm was obtained to reduce the population of *E. coli* O157:H7 on lettuce for 1.2 to 1.5 log CFU/g at 1 min, comparing to deionized water only 0.6 to 0.8 log CFU/g. There were no significant differences ($P \geq 0.05$) between NEW at 89 ppm and standard hypochlorite treatment (SH) at 100 ppm for their bacterial activities on reducing pathogenic bacteria on lettuce, and the result also depicted NEW was more sensitive to *Salmonella* rather than *E. coli* O157:H7.

Surface problems

Singh et al. (2003) reported that greater reductions of *E. coli* O157:H7 were observed with baby carrots (0.54-, 1.06-, and 1.39 log CFU/g) in comparison to shredded lettuce (0.26-, 0.73-, and 0.76 log CFU/g) by using 5-, 10-, or 20 mg/l aqueous chlorine dioxide (ClO_2) for 15 min, respectively. The bacteria cells are prone to adhere tenaciously to shredded lettuce surface as

compared to baby carrots. The attachment of *E. coli* O157:H7 affected by the different properties of the surfaces was also confirmed by Han et al. (2000) on green peppers. The injured sites on vegetable surfaces provide enough nutrients and moisture for bacteria attaching and growing, and moreover, these sites will protect bacteria from sanitation (keskinen et al., 2009).

Accounting for several factors throughout production and postharvest handling, the microbial ecosystem on the surface of vegetables is complex. Without considering extrinsic factors such as how the presence and numbers of microorganisms differ, weather conditions or agricultural practices differ, geographical areas of production differ. One explanation for the great variation of sanitizers in the disinfection of food produce is the different characteristics of produce. Sanitizers have been investigated for the abilities to remove all microorganisms on the surface of produce associated with the multilayered hydrophobic cutin covered on vegetable surfaces, bruised and cut surface tissues, diverse surface morphological structures, and colonization and biofim development. The above information provide some aspects that have contributed to the efficacy of sanitizers on food products (Want et la., 2006; Burnett and Beuchat, 2001).

Fruits

Electrolyzed water is not only applied as an effective antimicrobial on vegetables, but also used on fruits. In one study on strawberries, strawberries (100 g) were dip treated in a sterile 600 ml beaker with electrolyzed water prepared from 0.05% or 0.1% NaCl solution (Udompijitkul et al., 2007). The ratio of samples and solution is 1:3 by weight and contact time applied was 5, 10 or 15 min washing with electrolyzed water generated using 0.1% NaCl brine

solution. The reduction of maximum indigenous bacteria with 1.44 to 2.23 log CFU/g when exposed to 10 min (Udompijitkul et al., 2007). Apple samples (60 g) inoculated with *E. coli* O157:H7, were dipped in a 600 ml solution of electrolyzed water (70 ppm) for 8 min, showed a reduction of 1.08 log CFU/cm^2 compared to other treatments (Wang et al., 2006).

Eggs

Several studies have shown electrolyzed water applied as an effective sanitizer against *Salmonella* on the surface of eggs. Cox et al. (1990) found that *Salmonella* can exist on 71% of eggshell, 80% of chick conveyor belts, and 74% of paper pad trayliners. Bailey (1998) demonstrated that although very small amounts of fertile eggs entering the hatchery carried *Salmonella*, the spread of this bacterium was really fast and extensive. Russel (2003) inoculated *Salmonella typhimurium, L. monocytogenes, Staphylococcus aureus,* and *E. coli* on eggs (15 x 4 repetitions) to determine the effect of electrolyzed water (EW). Each sample was sprayed with EW at 8 ppm FAC with a pH of 2.1 through two electrostatic spray nozzles for 15 sec each hour for 24 hours. Electrolyzed water was proved to completely eliminate 53.3% of *Salmonella typhimurium, 93.3% of L. monocytogenes, 80% of Staphylococcus aureus,* and 100% of *E. coli* from the surface of 15 eggs in repetitions 1, 2, 3, and 4, respectively. The results showed that EW was effective to eliminate above pathogenic bacteria from hatching eggs with electrostatic spraying.

Poultry

Because a number of outbreaks related to the consumption of poultry were associated with *Campylobacter jejuni*, chlorine rinses are commonly used during processing for pathogen reduction (James et al., 1992; White et al., 1997). The use of some chemical antimicrobials has resulted in having chemical residues, discoloration of carcasses, and having a high cost and limited effectiveness. Researchers have looked for other means to show effectiveness against *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *L. monocytogenes* (Venkitanarayanan et al., 1999). Park et al. (2002) worked with EW and chlorine water (25 and 50 mg/l residual chlorine) on chicken wings by using dipping method to determine the reduction of a six-strain mixture of *C. jejuni* population (7.5 log CFU/ml). With 25 mg/l, EW was more effective than chlorine water on inactivation of *C. jejuni*, and the population of *C. jejuni* was undetectable with both treatment solutions at 50 mg/l for 10 sec.

Seafood

Vibrio parahaemolyticus is a halo tolerant bacterium that can be isolated from a variety of seafood, including codfish, sardine, clam, shrimp, scallop and oyster (Liston, 1990). It caused three outbreaks of 425 cases of gastroenteritis related to consumption of crabs without proper cooking in Maryland (Molenda et al., 1972). Mahmoud et al. (2004) performed studies that examined filleted carp immersed in 10-fold volume of sterile deionized water, anodic solution [EW(+)], cathodic solution [EW(-)], and cathodic followed by anodic solutions [EW(-)/EW (+)] and inoculated 15 strains of bacteria isolated from carp to test the efficacy of EW. Mahound et al. (2004) also confirmed the efficacy of the disinfection properties of EW. By dipping whole and filleted carp in EW (+), they reduced the total population of aerobic bacteria by 2.8 and 2.02 log

units at 15 min, respectively. The authors concluded that the efficacy of different treatments followed the order: EW (+) > EW (-)/EW (+) > EW (-) > deionized water. Therefore, there are a number of studies indicating that EW can inactivate contaminating microorganisms on seafood prevent spoilage, and extend shelf life (Mahmoud, et al., 2004).

One of the problems observed in the examination of various studies is a lack in consistency in the application of EW to standardized quantities of products. In our subsequent testing of the efficacy of EW on produces, we hope to achieve some sense of standardizing the level of target surface area in order to better compare results between different product applications.

CHAPTER III

METHODOLOGY

Electrolyzed water generation

Electrolyzed water was generated using an EcaFlo080 electrolyzed water generator supplied and manufactured by Integrated Environmental Technologies, Inc (Little River, SC) supplied by SanAquel LLC (Unitherm Food Systems Inc.) and used at Oklahoma State University. Electrolyzed water was generated at 19 to 21 amps with 23% brine injection at a pH of approximately 6 to 6.5 by modifying the generator conditions during production. The pH and oxidation reduction potential (ORP) were adjusted by brine flow adjustment on the electrolyzed water generator. Generally, electrolyzed water was generated at 200 to 260 ppm free available chlorine (FAC), stored in a sterile plastic container, and then diluted to a preferred FAC level for and intended study using distilled water on the day of the experiment. Samples to be used for the determination of pH, ORP, and FAC were also collected.

Determination of pH, ORP, and free available chlorine (FAC)

The pH and ORP of the EW were measured with an AR15 pH and ORP meter (Accumet Research, Fisher Scientific Co., Pittsburgh, PA, USA) while the free available chlorine content of electrolyzed water was measured using a Hach DPD-FEAS digital titrator method (Hach, 8210,

Loveland, CO) as described by the manufacturer. In brief, 1 ml of electrolyzed water was added to 99 ml of deionized water, and a 25 ml sample of this diluted electrolyzed water was transferred to an Erlenmeyer flask. A DPD-Free chlorine powder pillow was added to the sample with swirling and shaking. Then, the sample was titrated using 0.00564 N ferrous ethylenediammonium sulfate (FEAS) until colorless. The display on the digital titrator indicated the free chlorine concentration in milligrams per liter (mg/l). The treatment solutions were prepared before each experiment then stored and used within 1 h.

Electrolyzed water dipping treatment

Vegetable preparation

Fresh carrots (*Daucus carota* L. *cv*. sativus), grape tomatoes (*Lycopersicum esculentum* Mill), cabbage (*Brassica oleracea* L.), peppers (*C. annuum*), and spinach (*Spinacia oleracea* L.) were purchased from a local Wal-Mart in Stillwater, Oklahoma, United States. The produce was transported to the laboratory, sealed individually in a sterile plastic bag, and stored at room temperature (22 ± 2^{o}C) for 48 h. A sanitized knife was used to cut the carrots into cylinders (20-35 mm dia and 30 - 40 mm thick). The wrapper leaves of the cabbage and spinach were discarded, and the intact samples were cut into medium sized pieces and the petiole was trimmed. A few drops of grape tomatoes juice were squeezed on grape tomatoes to enhance surface growth of indigenous bacteria during a short per-trial storage period. Grape tomatoes and peppers were sealed in sterile bags and held at room temperature.

Surface area

This experiment was conducted with the intention of using a standardized surface area (150 cm^2) of food produce for determining the efficacy of different sanitizers. Carrots and baby carrots were calculated as $S = 2\pi r^2+2\pi rh$; grape tomatoes were calculated as $S = 4\pi r^2$; cabbage and spinach were calculated as S = ab; jalapeño peppers were calculated as $S= 2\pi r^2+2\pi rh$. The surface areas of each of five vegetable samples were calculated and approximately 150 cm^2 of product was then distributed into sterilized baskets.

Dipping study using electrolyzed water at three different concentrations and three different dwell times

Dipping treatments were performed in 1000 ml of treatment solution for 1, 2, and 4 min with agitation. Electrolyzed water used for carrots, grape tomatoes, cabbage, peppers, and spinach was diluted to approximate FAC levels of 50, 100, and 200 ppm. Samples were separately immersed in tap water (FAC less than 5 ppm) and EW (FAC levels of 50, 100 and 200 ppm) with agitation. Samples were held in sterile baskets which could be totally immersed in 1000 ml of solution. Each assay was run in 1000 ml of solution and performed in quadruple replication. Once treated with EW solutions, samples were drained and then transferred to sterile bags. Samples diluted in 50 ml 0.1% of buffer peptone water (BPW) were stomached to resuspend remaining viable cells. Serial dilutions were made in 0.1% BPW and plated on PCA agar in duplicate. Untreated control samples were included to verify the initial level of indigenous microorganisms

on carrots, grape tomatoes, cabbage, peppers and spinach. Plates were incubated for 48 h at 30^{o}C and colony counts were recorded.

Antimicrobial activity on grape tomatoes when electrolyzed water is replenished during treatment

Electrolyzed water treatments for grape tomatoes were done at FAC levels of approximately 50, 100, and 200 ppm. Each treatment time was equally divided into 3 sub- periods, in which we immersed test products into fresh solutions of EW for comparison with our other study where the entire length of time was spent in the same original solution. In order to examine the difference between EW with, or without, replenished solutions for the same dipping time, the treatment time of each set was divided into 1 min (20 sec + 20 sec + 20 sec), 2 min (40 sec + 40 sec + 40 sec), and 4 min (80 sec + 80 sec + 80 sec) periods. For example, for 1 min treatment, samples would be immersed in one beaker for 20 sec, and then sequentially transferred to a second beaker for 20 sec, and then into a third beaker for the final 20 sec. Samples with 150 cm^2 surface area were also separately immersed in tap water (FAC less than 5 ppm). Samples were processed in this manner using electrolyzed water at 50, 100, and 200 ppm FAC with agitation. Each assay was run in 1000 ml of solution and performed in quadruple replication. Once treated with antimicrobials, samples were transferred to sterile bags and diluted with 50 ml of 0.1% BPW for stomaching and resuspension of remaining microorganisms. Serial dilutions were made in 0.1% BPW and plated on PCA agar in duplicate. Untreated control samples were included to verify the initial level of enumeration of indigenous microorganisms on grape tomatoes. Plates were incubated for 48 h at 30^{o}C and colony counts were recorded.

Effect of Lactic acid on different vegetables

Lactic acid was prepared from 88% concentrations to 1%, 2%, and 4% solutions diluted in sterile distilled water before each treatment. Baby carrots and grape tomatoes were separately immersed in 1000 ml of 1%, 2%, and 4% lactic acid and in tap water. Samples were held in sterile baskets which could be totally immersed in 1000 ml of solutions. Each assay was run in 1000 ml of solution and performed in quadruple replication. Once treated with lactic acid solutions, samples were drained and then transferred to sterile bags. Samples diluted in 50 ml 0.1% of buffer peptone water (BPW) were stomached to resuspending remaining viable cells. Serial dilutions were made in 0.1% BPW and plated on PCA agar in duplicate. Untreated control samples were included to verify the initial level of indigenous microorganisms on baby carrots and grape tomatoes. Plates were incubated for 48 h at 30^{o}C and colony counts were recorded.

Effect of peroxyacetic acid on baby carrots and grape tomatoes

Peroxyacetic acid (PAA) (15%, v/v) was prepared and diluted in sterile distilled water before each treatment. In our experiment, we used 50 ppm PAA solution (1 ppm = 0.0001%). Baby carrots and grape tomato samples were held in sterile baskets which could be totally immersed in solution. Each assay was run in 1000 ml of solution and performed in quadruple replication. Once treated with antimicrobials, samples were transferred to sterile bags. Samples

diluted in 0.1% BPW were stomached to resuspending remaining viable cells. Serial dilutions were made in 0.1% BPW and plated on PCA agar in duplicate. Untreated control samples were included to verify the initial level of indigenous microorganisms on baby carrots and grape tomatoes. Plates were incubated for 48 h at 30^{o}C and colony counts were recorded.

Effect of the combination of lactic acid and electrolyzed water on vegetables

Two combination sanitizing solutions (0.5% lactic acid plus EW 25 ppm, and 1% lactic acid plus EW 50 ppm) were used on grape tomatoes and baby carrots. Each assay was run in 1000 ml of solution and performed in quadruple replications. Baby carrots and grape tomato samples were dipped in the solutions for 1, 2, and 4 min, respectively. Samples were held in sterile baskets which could be totally immersed in 1000 ml of solutions. Each assay was run in 1000 ml of solution and performed in quadruple replication. Once treated with antimicrobials, samples were transferred to sterile bags. Samples diluted in 0.1% BPW were stomached to resuspending remaining viable cells. Serial dilutions were made in 0.1% BPW and plated on PCA agar in duplicate. Untreated control samples were included to verify the initial level of indigenous microorganisms on baby carrots and grape tomatoes. Plates were incubated for 48 h at 30^{o}C and colony counts were recorded.

Evaluation of enhancement of antimicrobial effect provided by sodium thiocyanate on baby carrots previously treated with electrolyzed water

The enhancement of antimicrobial effect was examined by various spray applications of 100 mM sodium thiocyanate on products previously treated with electrolyzed water at 50, 100, and 200 ppm FAC. Electrolyzed water was diluted to working concentrations (50, 100, and 200 ppm) from higher concentrations by addition of sterile deionized water. Baby carrots, equivalent to approximately 150 cm^2 in surface areas, were immersed in EW solutions for 1 min, allowed to drain for 5 min, and then spray processed with 100 mM sodium thiocyanate (test samples) or 0.1% BPW (control samples), and then processed for microbial counts (stomaching in 0.1% BPW, making appropriate dilutions in 0.1% BPW, and plating in duplicate on PCA). Additional treatments included various hold times after sodium thiocyanate spray treatment (1, 2, and 4 min) before final microbial processing. Additional controls included untreated baby carrots to establish the initial level of indigenous microorganisms on baby carrots. All treatments were done in quadruplicate replication.

Statistical analyses

All experiments were performed in quadruple replications. Samples were serially diluted and plated in duplicate for each analysis. The resulting data were analyzed using a one way analysis of variance (ANOVA) to determine the level of significance between the effect of different sanitizers, types of vegetable, and treatment times. Pairwise multiple comparisons were then completed for each using the Holm-Sidak method. A *P* value of 0.05 was set for the level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

Effect of dipping times on bactericidal efficiency of tap water and different concentrations of EW

A number of chemical antimicrobials have been tested with various degrees of effectiveness, among which electrolyzed water (EW), with its convenient method of generation, has been shown to be a promising disinfectant technology for the food industry. In our research, we examined the effectiveness of EW as an antimicrobial rinse treatment on fresh and fresh-cut vegetables compared with several other antimicrobials at different dwell times, and standardized against a common surface area.

Effect of EW on five types of fresh vegetables at three different concentrations and three different dwell times.

Applications of chlorinated water of 50 to 200 ppm FAC are widely used on fresh produce to reduce bacterial contamination in the food industry (Beuchat et al., 1998). Studies have shown that some chemical disinfectants can be differentially effective against various types of natural microorganisms on fresh produce. Our experiments were designed to evaluate the inactivation of indigenous bacteria associated with different types of fresh produce using EW at

50, 100, and 200 ppm FAC. In each of the following figures, data is presented both as "log reduction" directly obtained with the treatment (panel A) and as a "relative effect" that is relative to the total bacterial population (panel B) which may be different from one product to another.

The effects of dipping time (1, 2, and 4 min) on the reduction of indigenous bacteria associated with carrots, grape tomatoes, cabbage, jalapeño pepper, and spinach leaves treated with EW (50, 100, and 200 ppm) at room temperature (22 ± 2°C) against a standardized surface area of product (150 cm^2) was examined. For a 1 min dip treatment, the log reduction in bacterial count obtained with EW at 50, 100, and 200 ppm FAC was 1.07, 1.24, and 1.3 log CFU/ml, respectively (carrots); 1.01, 1.67, and 1.88 log CFU/ml, respectively (grape tomatoes); 2.86, 3.62, and 3.74 log CFU/ml, respectively (cabbage); 0.53, 1.5, and 1.66 log CFU/ml, respectively (pepper); 1.26, 1.94, and 3.04 log CFU/ml, respectively (spinach) (Fig. 1A). The relative influence of inactivation by EW observed was 200 ppm > 100 ppm > 50 ppm. Electrolyzed water at 200 ppm FAC showed a significant difference ($P < 0.05$) compared to 100 ppm on four types of vegetables with the exception of carrots. Cabbage leaves showed a higher log reduction ($P < 0.05$) in natural bacteria than other vegetables with only a 1 min dipping time. Similar results were observed when displayed as the proportion of total bacteria, or the relative reduction of total bacteria on cabbage (Fig. 1B). Rinsing carrots with either tap water or EW at 50 ppm did not show much difference for 1 min of dipping time, and the same results observed that relative reduction of natural bacteria on carrots by washing with tap water or EW at 50 ppm.

Vegetables, such as cabbage and spinach, may have a unique tissue structure that provides surface bacteria food contact with solution compounds from electrolyzed water. These results are similar to the findings discovered by Izumi (1999), who also indicated that microbes on the surface of tissues were more greatly reduced by electrolyzed water than in a macerated matrix. Guentzel et al. (2008) reported a similar study in which they used dip treatment (10 min) for green leafy vegetables with electrolyzed water (100–120 ppm FAC) and obtained a reduction

of 4.0-5.0 log CFU/ml of five bacteria strains tested. Guentzel et al. (2008) also suggested that the population of surviving bacteria on vegetables could be attributed to insufficient contact time, structural characteristics of vegetable surfaces (smooth and rough surfaces), and various protective mechanisms of adhesive microbial biofilms.

Results in our studies indicated that EW at 200 ppm FAC was more effective ($P < 0.05$) than that of tap water or either 50 or 100 ppm FAC in reducing populations of indigenous bacteria associated with five different types of vegetables for 1 min (Fig. 1). As Venkitanarayanan et al. (2002) and Guentzel et al. (2008) observed in their studies, they suggested that the short time of exposure to chemical agents may allow the survival of bacteria on produce and prolonging the contact time with antimicrobials should be able to increase the effectiveness of EW. In order to compare the efficacy of EW at 50, 100, and 200 ppm FAC and tap water, this study was further conducted to use the same treatment methods, but with longer treatment times to examine what effect this would have on indigenous bacteria associated with vegetable surfaces.

After dipping times were increased to 2 min (Fig. 2) and 4 min (Fig. 3), the microbial populations were significantly reduced on all types of vegetables when processed for 2 min, relative to rinse treatments with tap water ($\leq$ 5 ppm FAC). Electrolyzed water showed excellent performance on cabbage leaves with different concentrations and dipping times (Figs. 2A and 3A). The relative reduction of indigenous bacteria on cabbage was higher than other types of vegetables for 2 and 4 min dipping times, suggesting that cabbage provided more surface bacterial contact with compounds from electrolyzed water (Figs. 2B and 3B). No significant differences ($P > 0.05$) were found in the reduction of indigenous bacteria associated with some treatments washed with tap water vs. unwashed controls, indicating that reduction by physical rinse displacement is not likely the major factor in the observed reductions we obtained. This fact demonstrates that for many types of vegetables, the population of bacteria on fresh produce surfaces will remain fairly high even after being rinsed with tap water for 4 min (Fig. 3A).

In all, the main aim of this study was to assess the effectiveness of EW on a) standardized surface areas of vegetables, at b) different concentrations, and c) different processing times compared to regular tap water rinse treatment and unwashed controls. The greatest log reduction and relative reduction was achieved with EW at 200 ppm FAC on cabbage leaves at 4 min (Fig. 3). Free available chlorine may be considered as the main contributor to the bactericidal activity of electrolyzed water. Izumi (1999) evaluated disinfection of vegetables by EW. The total native bacteria on fresh-cut carrots, bell peppers, and spinach were reduced by 0.6 to 2.6 log CFU/g by dipping in EW (20 ppm FAC). It was also proven that EW at 50 ppm FAC had a better bactericidal effect than 15 or 30 ppm with the same treatment and times. The higher level of EW (200 ppm FAC) produced a high log reduction than the two lower levels in reducing indigenous bacteria from the surfaces of vegetables. In our study, the concentration of EW less than 200 ppm FAC for 1 min treatment was sufficient for the disinfection of fresh vegetables, but greater concentrations and processing times may provide even greater reductions (Figs. 2A and 3A). Compared to other vegetables, the relative effect of different sanitizers on five types of vegetables with the same dipping times demonstrated that electrolyzed water had a better effect on cabbage (Figs. 2B and 3B). Adams et al. (1989) and Deza et al. (2003) recommended that EW at 100 ppm FAC should be considered the upper limit of the working concentration in the food processing industry, not only due to its effective performance on reducing bacteria on fresh vegetables, but also due to limited effect on the produce tainting as well as equipment corrosion that result from higher levels of chlorine. Thus, EW at 50 ppm FAC was chosen for the following treatments to compare its effectiveness with other antimicrobials.

Antimicrobial activity on grape tomatoes when electrolyzed water is replenished during treatment

Hypochlorous acid, the most effective component of EW, is a very reactive oxidizing agent which can decrease dramatically after interaction with organic material in foods. In our previous studies (Figs. 1, 2 and 3), there was no significant difference between EW at 50 or 100 ppm FAC on grape tomatoes at all treatment times. Because they were perceived to have minimal reactive organic material due to their unique smooth surfaces, grape tomatoes were chosen for testing the antimicrobial activity of EW that was replenished during the treatment time. Yang et al. (2003) suggested that the ability of EW to provide better microbial reductions is attributed to its ability to be freshly produced and used immediately. Brackett (1992) also pointed out that washing vegetables and fruits with the same recycled water may introduce and spread contaminants over produce by accumulating debris and thus increasing potential microbial populations.

In this experiment, each treatment time was equally divided into three dwell times whereby the EW solution was replaced with fresh EW during continued washing of the grape tomato samples (Fig. 4). After treatment with EW (200 ppm), the populations of indigenous bacteria on grape tomato surfaces were reduced by 3 log CFU/ml for 4 min when compared to washing with tap water, which only reduced bacterial loads less than 0.4 log CFU/ml (Fig. 4A). We also observed that there was no significant difference ($P > 0.05$) between replenished EW at either 50 or 100 ppm on grape tomato surfaces when washed for 1- or 2- min although significant differences were observed between EW at 50, 100, and 200 ppm FAC when rinsed for 4 min (Fig. 4A). Moreover, in comparison to our previous study on grape tomatoes processed for 1 min (Fig. 1), 2 min (Fig. 2), or 4 min (Fig. 3) without replenishing the EW solutions, we obtained greater reductions for the same concentrations and processing times when EW was replenished (Fig. 4). The replenished treatment caused more effective reduction of natural bacteria on grape tomatoes

for the same times and solutions (Figs. 1B, 2B, 3B and 4B). Therefore, the conclusion is, hypochlorous acid, the main contributor of bactericidal activity of EW, as it can be rapidly depleted due to oxidative reactivity, showed decreasing inactivation efficacy of EW as a sanitizer with time and can be more effective if replenished several times with fresh hypochlorous acid than if used continuously with one original solution.

Other antimicrobial treatments on fresh vegetables

Lactic acid

Lactic acid (LA) is approved as a substance that is "generally recognized as safe" (GRAS) by FDA for general or miscellaneous purpose of food usage (FDA, 1981). In our research, we examined dip treatments for grape tomatoes and baby carrots (as two different types of contact surfaces) using 1%, 2%, and 4% LA for 1, 2, and 4 min treatment times (Figs. 5 and 6). After the concentration of LA was increased from 1% to 2% and 4%, a significant ($P < 0.05$) reduction in the level of indigenous bacteria was obtained on both vegetables (Figs. 5A and 6A). Populations of natural bacteria were significantly ($P < 0.05$) reduced 1.09 log CFU/ml when grape tomato samples were dipped in 1% LA for 1 min, while baby carrots were only reduced 0.2 log CFU/ml with the same treatment time (Figs. 5A and 6A). For 1 min dipping time, the EW at 200 ppm showed a better relative effect on grape tomatoes than baby carrots (Figs. 5B and 6B). Treatment of grape tomatoes with lactic acid (Fig. 5A) showed higher log reduction at short treatment than did baby carrots (Fig. 6A), and higher log reductions with both increasing LA concentration and increasing treatment time. An appreciable increase in log reduction with baby carrots was only

observed with 4% LA (2 min) or 2% and 4% LA for 4 min (Fig. 6A). However, the best log reductions obtained with the longest treatment time (4 min) and highest concentration (4%) with baby carrots (< 2 log CFU/ml; Fig. 6A) did not approach those obtained with grape tomatoes (2-3 log CFU/ml; Fig. 5A). Microorganisms on grape tomatoes seem to be more sensitive to antimicrobials, possibly due to its smooth contact surface. We may conclude that lactic acid treatment assayed in this study could be effective in the reduction of natural microbial populations, dependent on vegetables, concentration, and treatment time.

Peroxyacetic acid

As a comparison to lactic acid, we examined the efficacy of another antimicrobial, peroxyacetic acid (PAA), used as a sanitizer on grape tomatoes and baby carrots for the purpose of reducing populations of indigenous bacteria (Fig. 7). Population reductions for indigenous bacteria on both types of vegetables, treated with 50 ppm PAA for as long as 4 min, were less than 1 log unit (Fig. 7A). Although significant ($P < 0.05$) reductions were obtained for grape tomatoes and baby carrots dipped in PAA solutions for 1, 2, and 4 min compared to tap water treatments and unwashed controls, there was no significant ($P > 0.05$) difference obtained between 1 and 2 min dip times on grape tomatoes (Fig. 7A). Several researchers have demonstrated the efficacy of PAA on reducing populations of *Salmonellae* and *E. coli* O157:H7 on cantaloupe and honeydew melon (Park and Beuchat, 1999). Perhaps a higher concentration, such as 80 ppm PAA as recommended by Hilgren and Salverda (2006) would have proved greater reduction of bacteria.

Lactic acid combined with electrolyzed water

We further tested the combination of lactic acid (LA) and EW (0.5 % LA + 25 ppm EW, 1% LA + 50 ppm EW) treatment on grape tomatoes and baby carrots for 1, 2, and 4 min. Combinations of chemical disinfectants may perform better in both the sensory and microbial quality of products (Rahman, et al., 2010), due to possible synergistic effects during treatment. Our results showed a significant difference ($P < 0.05$) with the combined treatment of lactic acid and EW (Figs. 8 and 9) as compared to lactic acid (Figs. 5 and 6) or EW (Figs.1, 2, 3, and 4) alone. For the combined treatment, we also noticed that the same concentration of the combined treatment seemed more likely to decrease indigenous bacteria populations on vegetables with smooth surfaces than with rough surfaces and with increasing treatment time (Figs. 8A and 9A), in which the antimicrobial activity of the combined solutions was more pronounced on grape tomatoes than baby carrots. For the same dipping time, there was more reduction of natural bacteria achieved by the higher concentration of combined solution on the same vegetable (Figs. 8B and 9B). Compared to what we observed with the combination of (1%) LA and EW (50 ppm) treatment (Fig. 9A), there were no differences observed for using a combination of (0.5%) LA and EW (25 ppm) in reducing microbial loads on baby carrots for 1, 2, and 4 min (Fig. 8A). The low reductions observed in these studies on baby carrots may again be due to its surface properties. From harvest to distribution, bacteria are firmly attached to indentations and natural irregularities on the intact food surface (Novak et al., 2003), and those with rough surfaces, such as baby carrots may play a role in repelling sanitizer on providing extensive sites for bacteria to attach, and become less accessible to antimicrobials.

Comparison of DI water, EW and sodium thiocyanate on the microbial flora of baby carrots at various treatment times

Sodium thiocyanate (ST) is part of the natural lactoperoxydase-thiocyanate-hydrogen peroxide (LP) system in milk that imparts antimicrobial activity to raw milk. This study was conducted to determine the bactericidal activity of sodium thiocyanate combined with EW, and more specifically, to examine what additional lethality may be obtained when sodium thiocyanate reacts with chlorine-containing disinfectant by products (DBP's) previously generated when hypochlorous acid reacts with organic material. When EW (50, 100, or 200 ppm FAC) solution alone was used to rinse baby carrots, total microbial loads were reduced by 1.33, 1.48, and 2.82 log CFU/ml, respectively (Fig. 10). When we examined the combination treatment of EW (50, 100, or 200 ppm) dip followed by sodium thiocyanate spray treatments, we obtained similar reductions of 1.3, 1.45, and 2.86 log CFU/ml, respectively (Fig. 10). This leads us to conclude that EW combined with sodium thiocyanate did not enhance the reduction on bacteria on baby carrots. We further modified the protocol to extend the contact time with sodium thiocyanate to 1, 2, and 4 min after treatment with EW. Compared to EW at 50 and 100 ppm, the use of EW at 200 ppm combined with sodium thiocyanate for all treatment times showed significant differences. The relative reduction of natural bacteria on baby carrots by treated with EW at 50 ppm or 100 ppm combined with sodium thiocyanate did not show any differences (Fig. 11). Although this extended time combination treatment did not provide any appreciable increase in log reduction with EW at 200 ppm FAC, we observed only a modest increase with EW at 50 and 100 ppm for 2 and 4 min extended contact times with sodium thiocyanate which may warrant additional studies to further evaluate this effect (Figs. 10 and 11).

Electrolyzed water has been approved to use on food products as an antimicrobial for many years due to its low cost and antimicrobial activity. In recent years, companies have built

new EW generators to facilitate automatic manufacture of EW solutions. This study concluded that EW with a slightly acidic pH showed bactericidal activity against indigenous bacteria on vegetable produce surfaces and were comparable to lactic acid and peroxyacetic acid. It is particularly effective for reducing microbial loads on fresh vegetable surfaces as a natural sanitizer in the food processing industry. On the other hand, it cannot be assumed that EW will completely eliminate indigenous bacteria associated with all types of vegetables. The bactericidal activity of EW on vegetables with firm "skins" (tomatoes, peppers, and cabbage) is more pronounced than those with more reactive surface properties and its efficacy may also differ depending on the rough or smooth surfaces of vegetables, such as carrots or baby carrots. Carrots may provide sites for bacteria to attach, become less accessible to antimicrobials, and may be more chemically reactive to the chlorine component in hypochlorous acid. Therefore, for food practices, using EW (50 ppm) with a 1 min treatment time may be effective in reducing microbial loads from some food produce surfaces whereas other types of produce may require higher levels and/or longer treatment times as identified in our study.

Our research suggests that EW can be utilized as a common sanitizer on fresh vegetables. Lactic acid was tested in combination with EW and performed well in reducing bacteria on food produce surfaces at lower levels of LA or EW than if either were used alone. All dipping solutions tested in our study were capable of reducing microbial populations to some extents, but, the antimicrobial effects were different dependent on concentration, treatment time, and type of produce. Hence, the application of EW technology in combination with other alternative antimicrobials for inactivating bacteria on fresh products should be considered and recommended. By standardizing the surface area of treatment, we are better able to determine the relative effectiveness on various types of produce because the main reactive area is the product surface. The data herein provides the foundation for further application of EW as a sanitizing agent in the vegetable processing industry. Therefore, future studies should be elucidated to validate these

findings for other antimicrobials combined with EW as well as the need for further studies with some specific strains of inoculated pathogens.

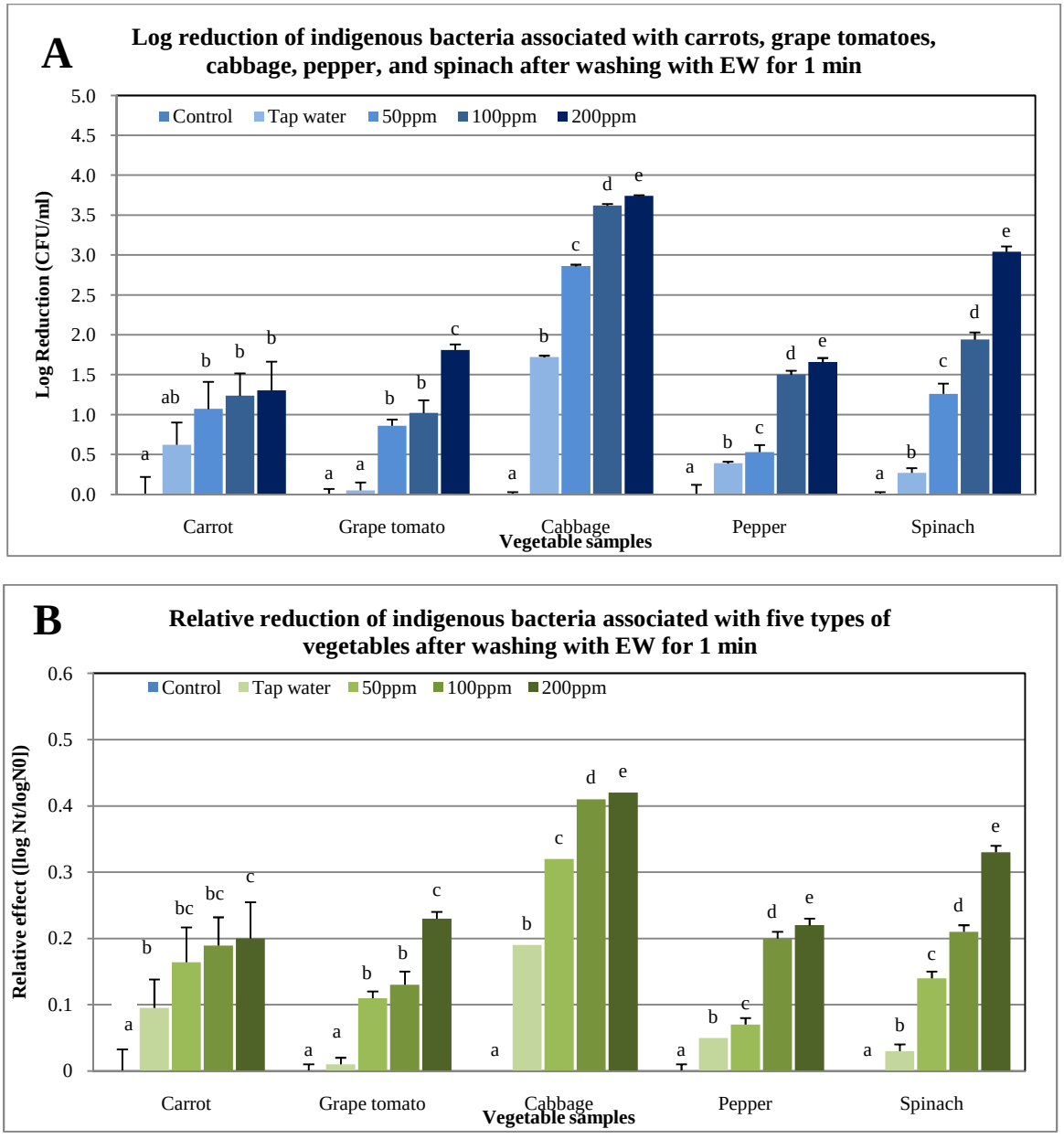


Figure 1. Carrots, grape tomatoes, cabbage, peppers, and spinach leaves were immersed into tap water or electrolyzed water (50, 100, or 200 ppm free available chlorine) for 1 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on five types of vegetable after washing with electrolyzed water for 1 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable

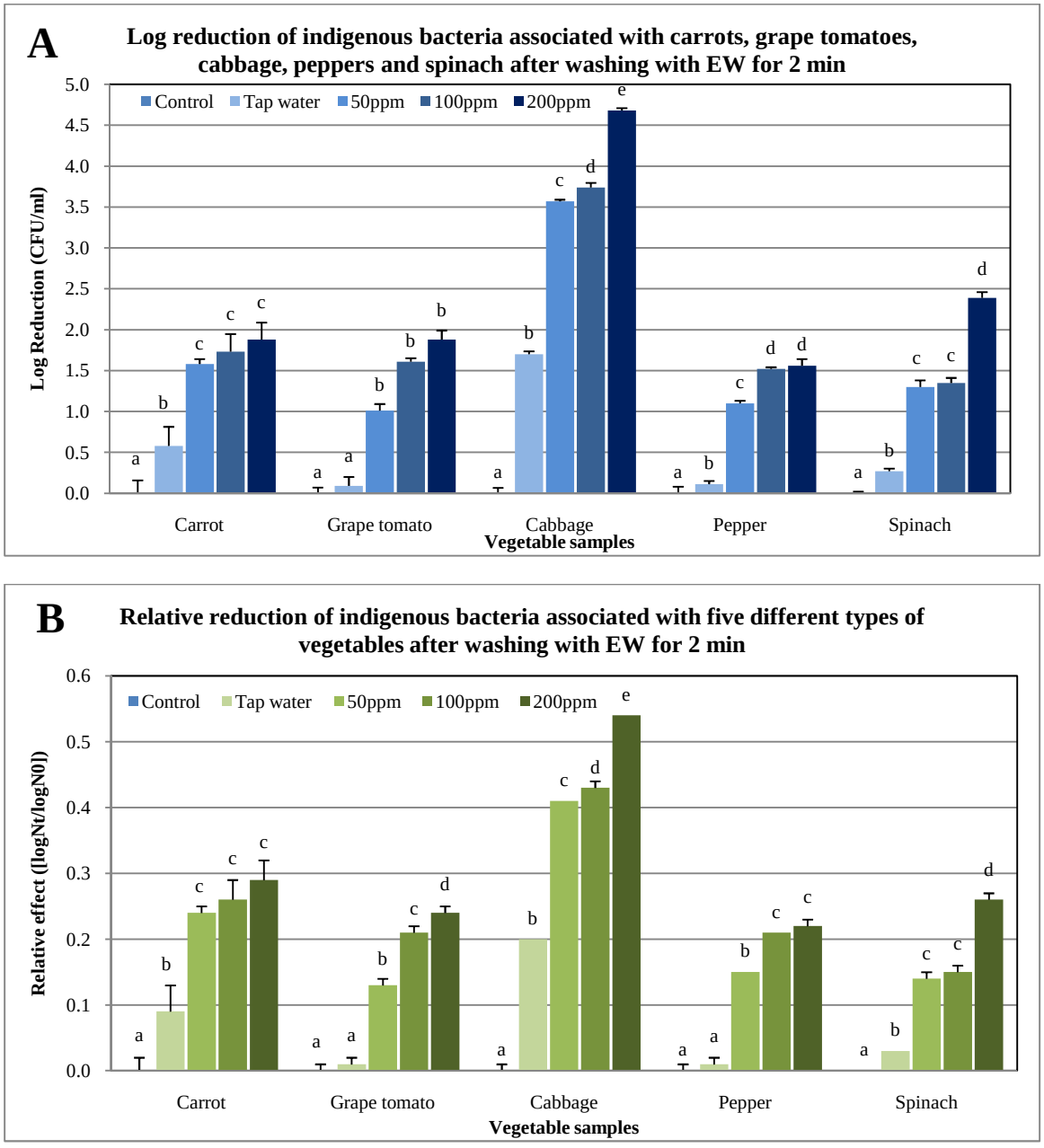


Figure 2. Carrots, grape tomatoes, cabbage, peppers, and spinach leaves were immersed into tap water or electrolyzed water (50, 100, or 200 ppm free available chlorine) for 2 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on five types of vegetable after washing with electrolyzed water for 2 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.

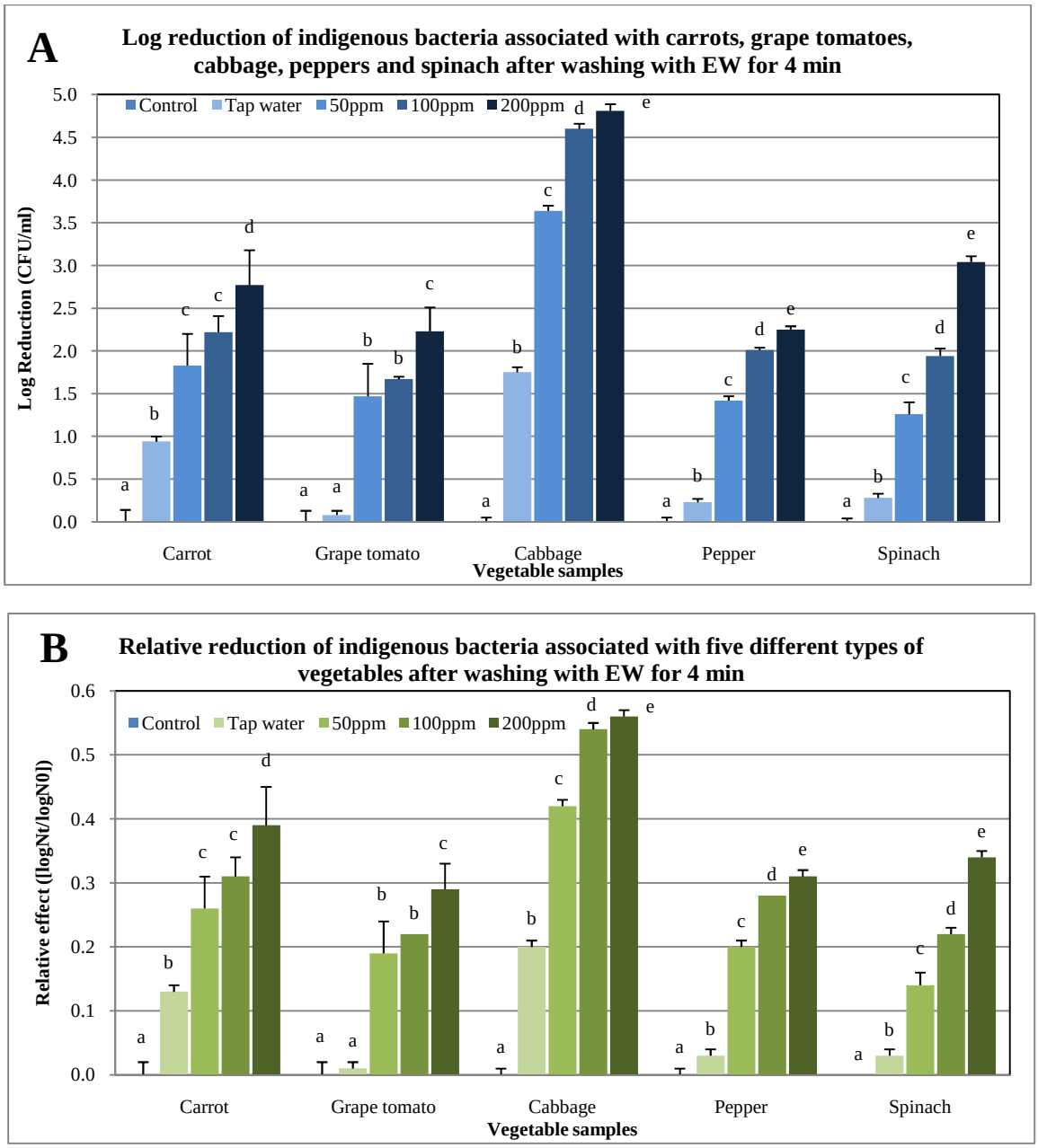
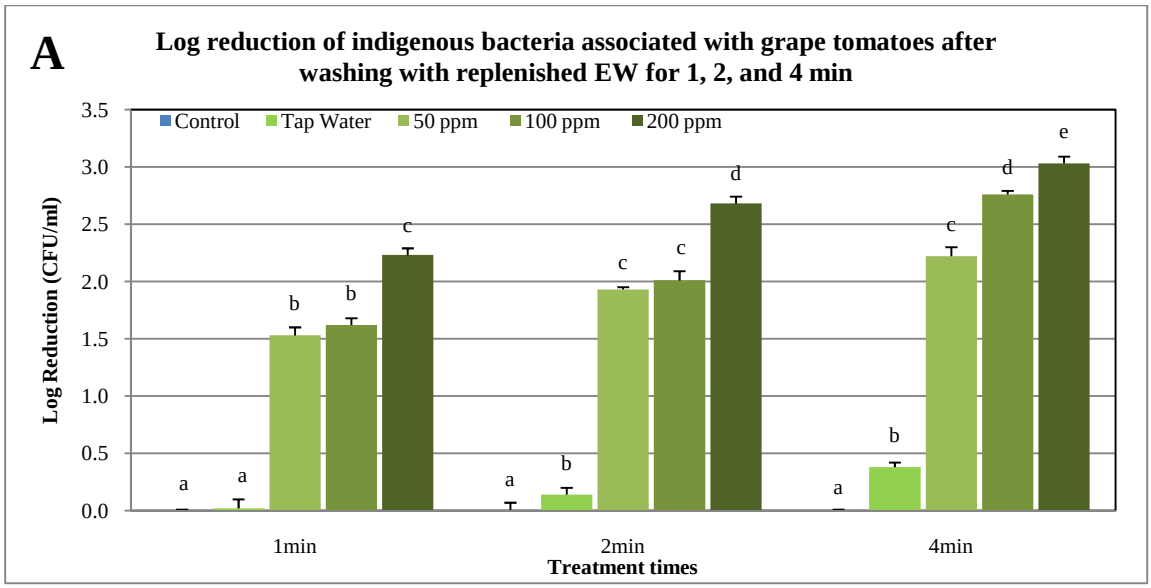


Figure 3. Carrots, grape tomatoes, cabbage, peppers, and spinach leaves were immersed into tap water or electrolyzed water (50, 100, or 200 ppm free available chlorine) for 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on five types of vegetable after washing with electrolyzed water for 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.



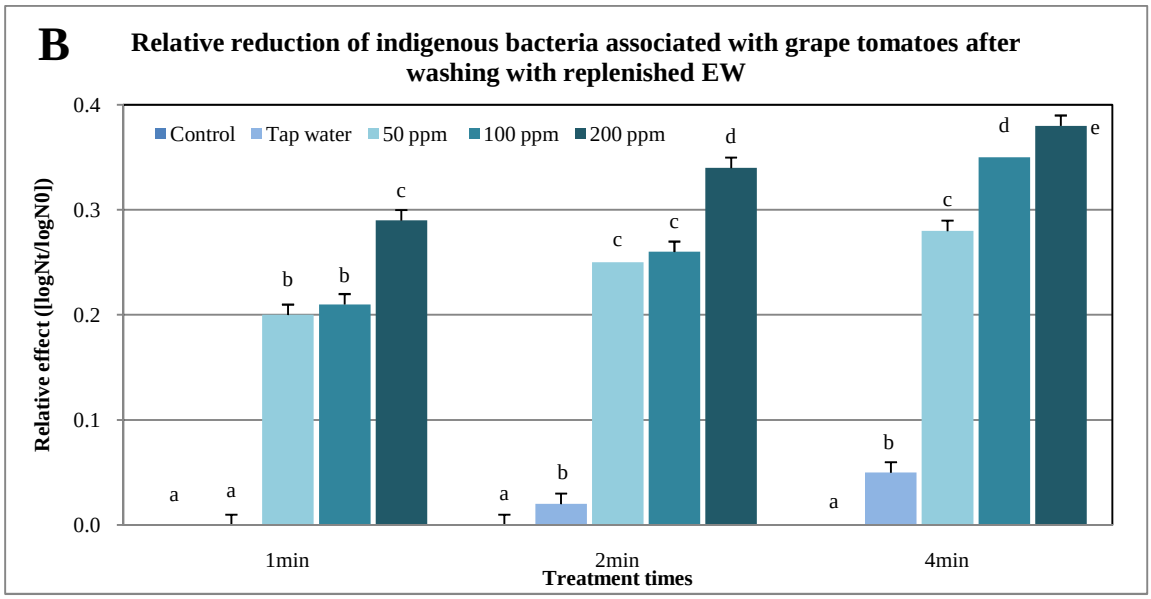


Figure 4. Grape tomatoes were immersed into tap water and replenished electrolyzed water (50, 100, or 200 ppm free available chlorine) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on grape tomatoes after washing with electrolyzed water for 1, 2, or 4 min. Each overall treatment time (i.e., 1 min) was split into 3 equal and shorter dwell times (i.e., 20 sec + 20 sec + 20 sec) whereby electrolyzed water solution was replenished for each short dwell time. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.

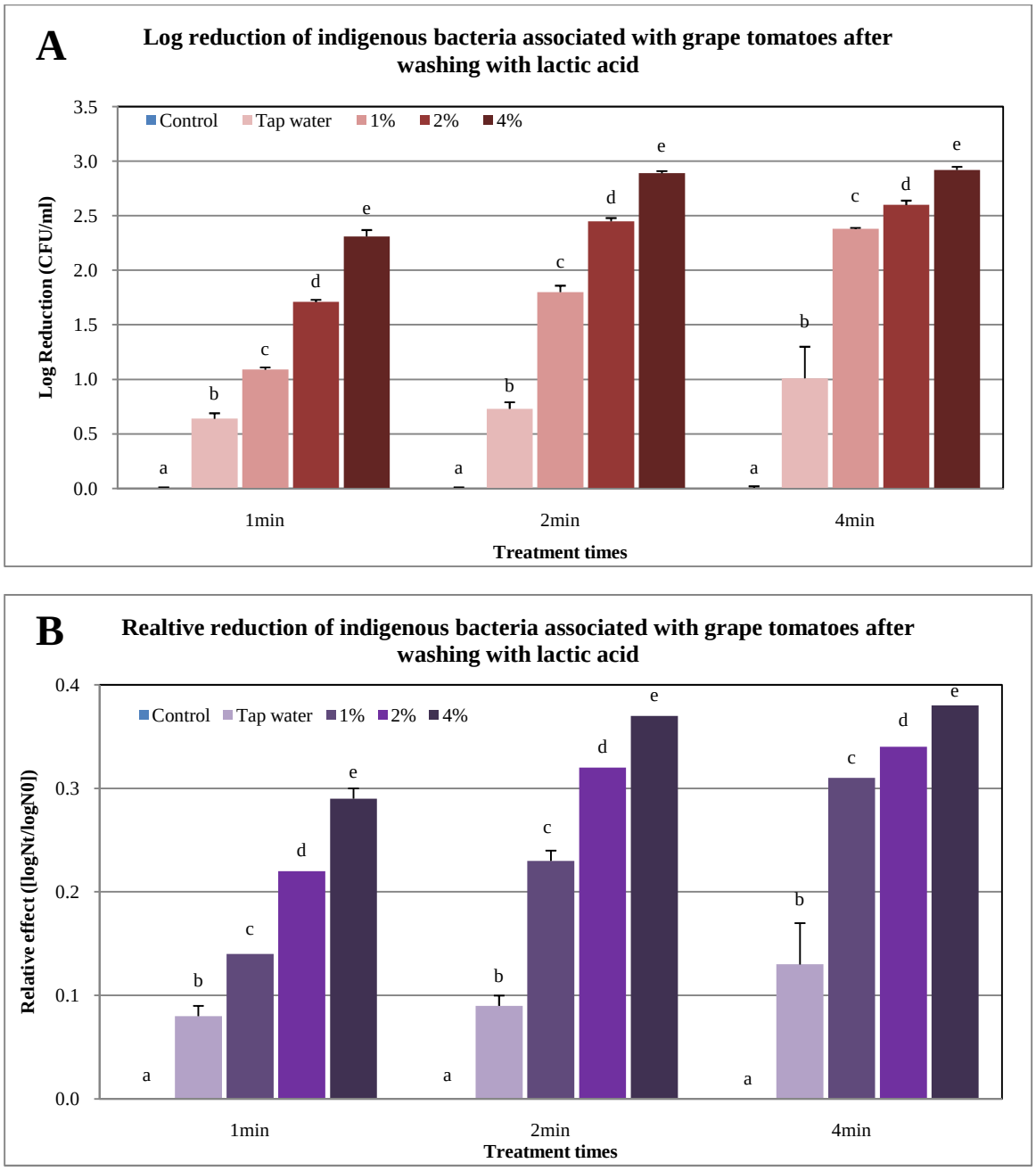


Figure 5. Grape tomatoes were immersed into lactic acid (1%, 2%, or 4%) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on grape tomatoes after washing with lactic acid for 1, 2, or 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.

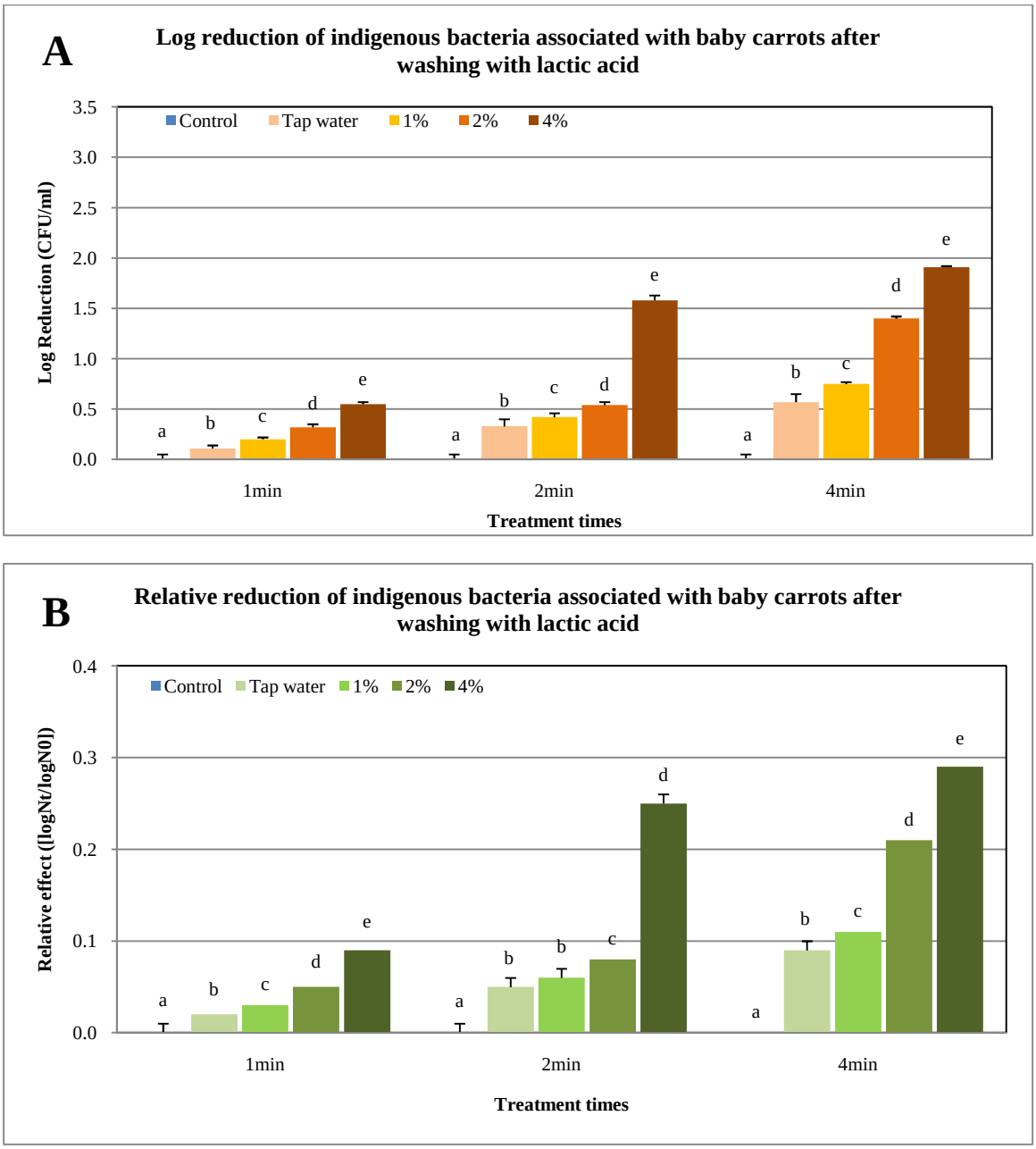
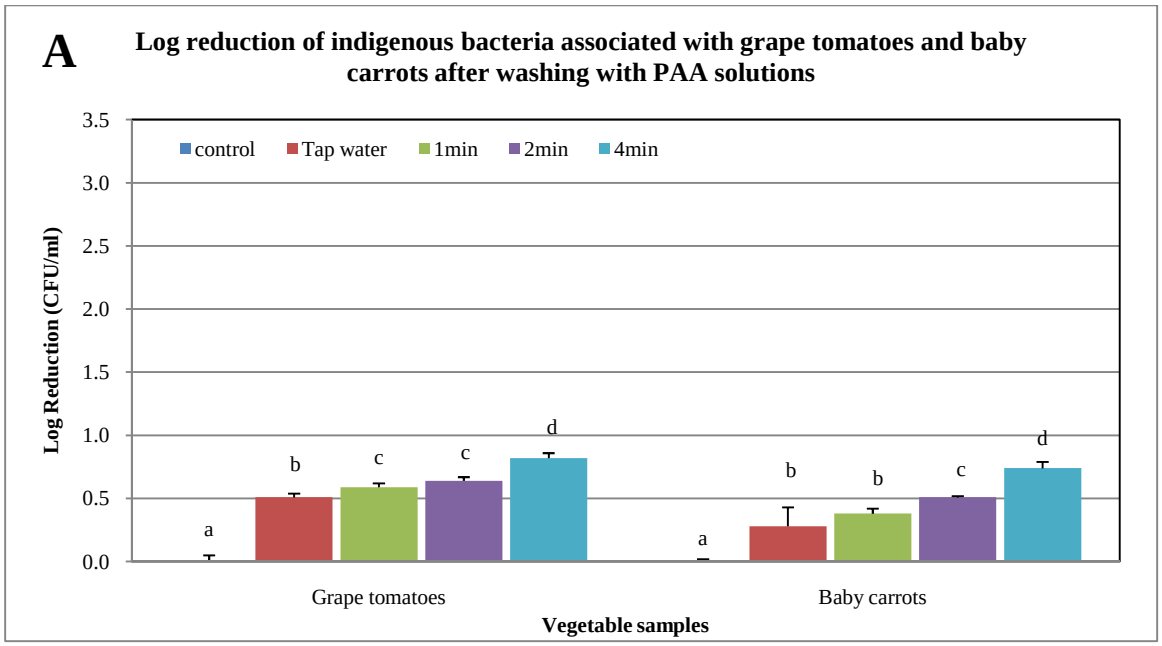


Figure 6. Baby carrots were immersed into lactic acid (1%, 2%, or 4%) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on baby carrots after washing with lactic acid for 1, 2, or 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.



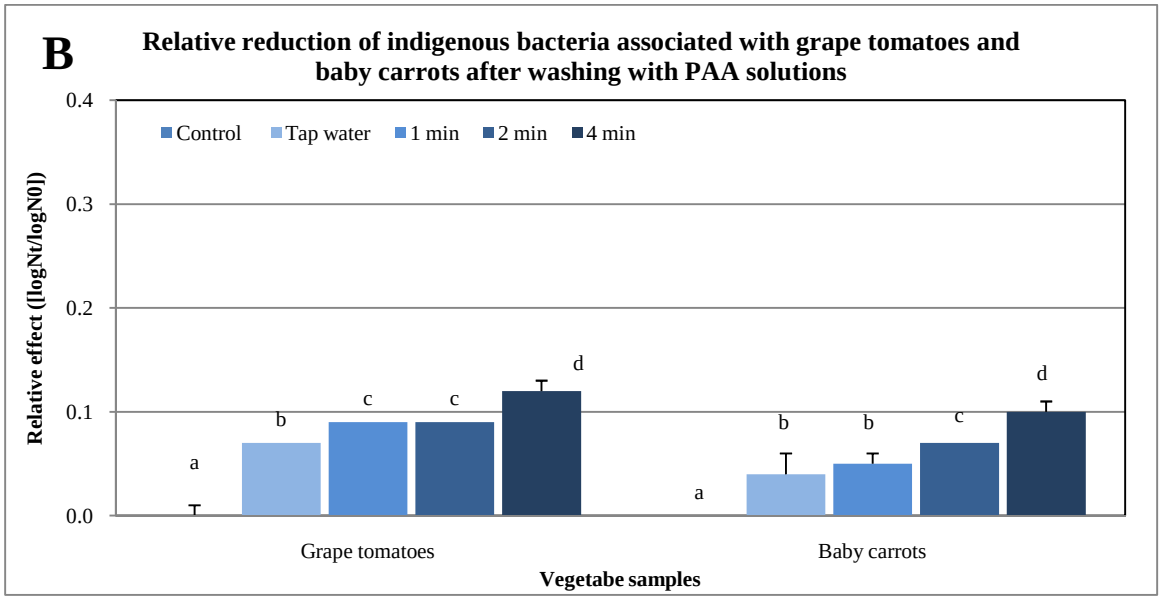
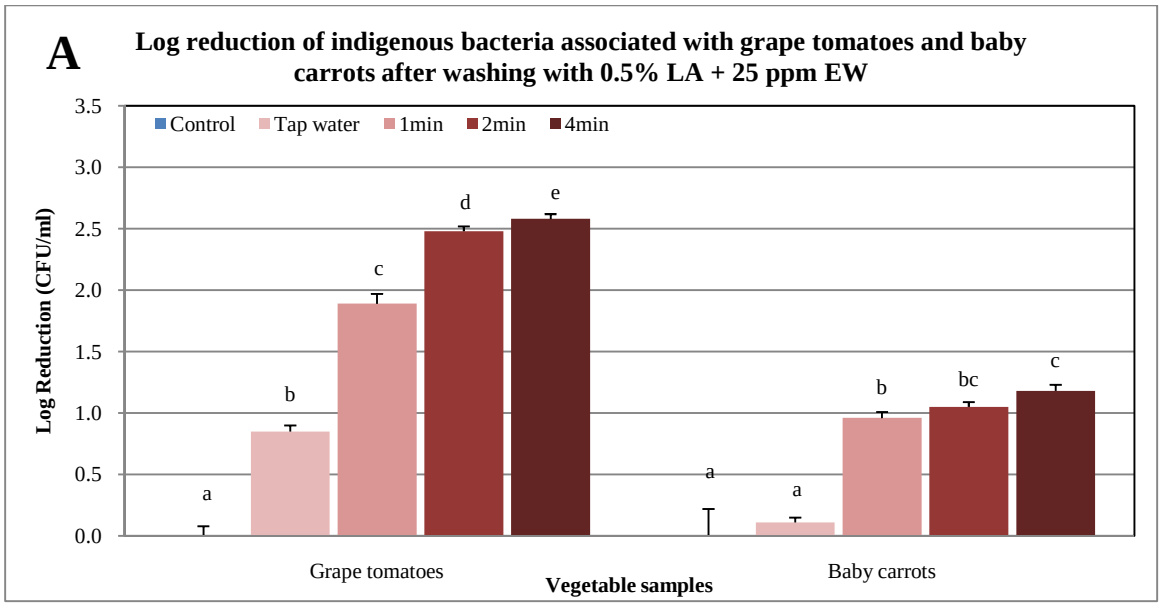


Figure 7. Grape tomatoes and baby carrots were immersed into peroxyacetic acid (50 ppm) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on grape tomatoes and baby carrots after washing with peroxyacetic acid for 1, 2, or 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.



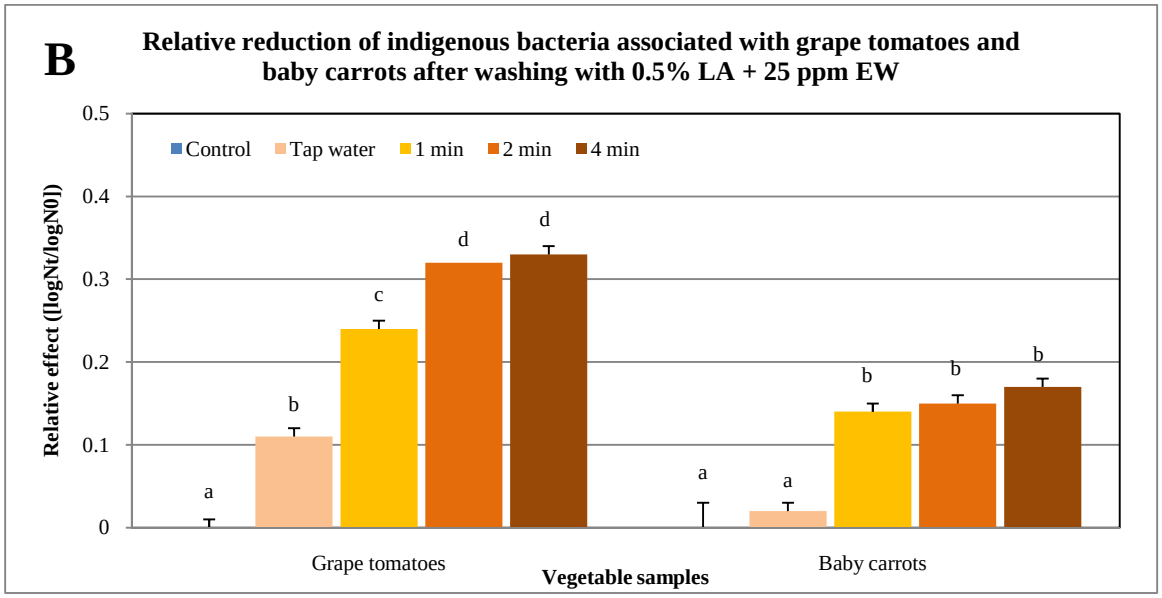


Figure 8. Grape tomatoes and baby carrots were immersed into the combination solution of lactic acid (0.5%) and electrolyzed water (25 ppm) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on grape tomatoes and baby carrots after washing with the combination solution for 1, 2, or 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.

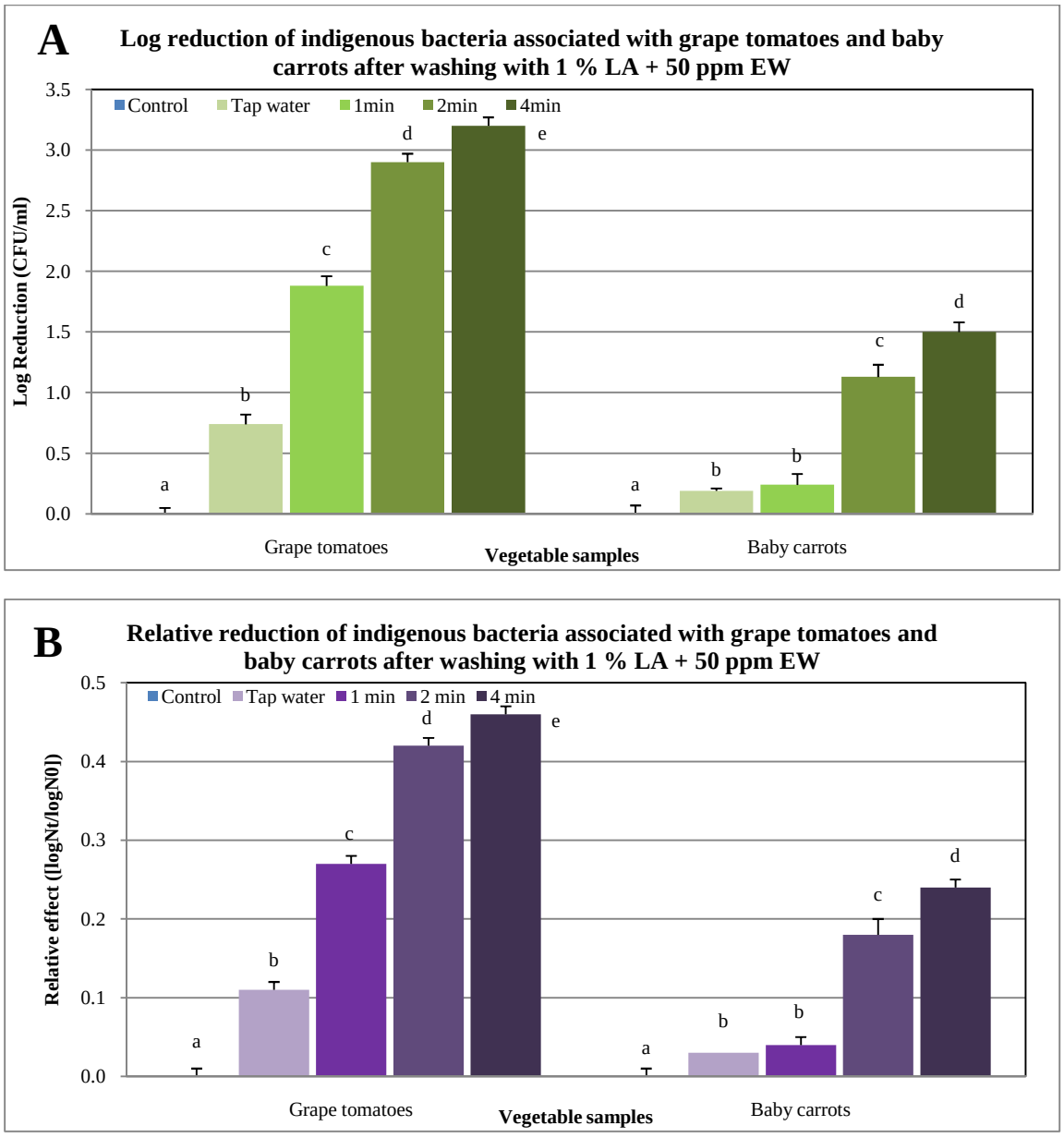


Figure 9. Grape tomatoes and baby carrots were immersed into the combination solution of lactic acid (1 %) and electrolyzed water (50 ppm) for 1, 2, or 4 min. Log reduction of indigenous bacteria (panel A) and relative reduction of bacteria (panel B) on grape tomatoes and baby carrots after washing with the combination solution for 1, 2, or 4 min. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same vegetable.

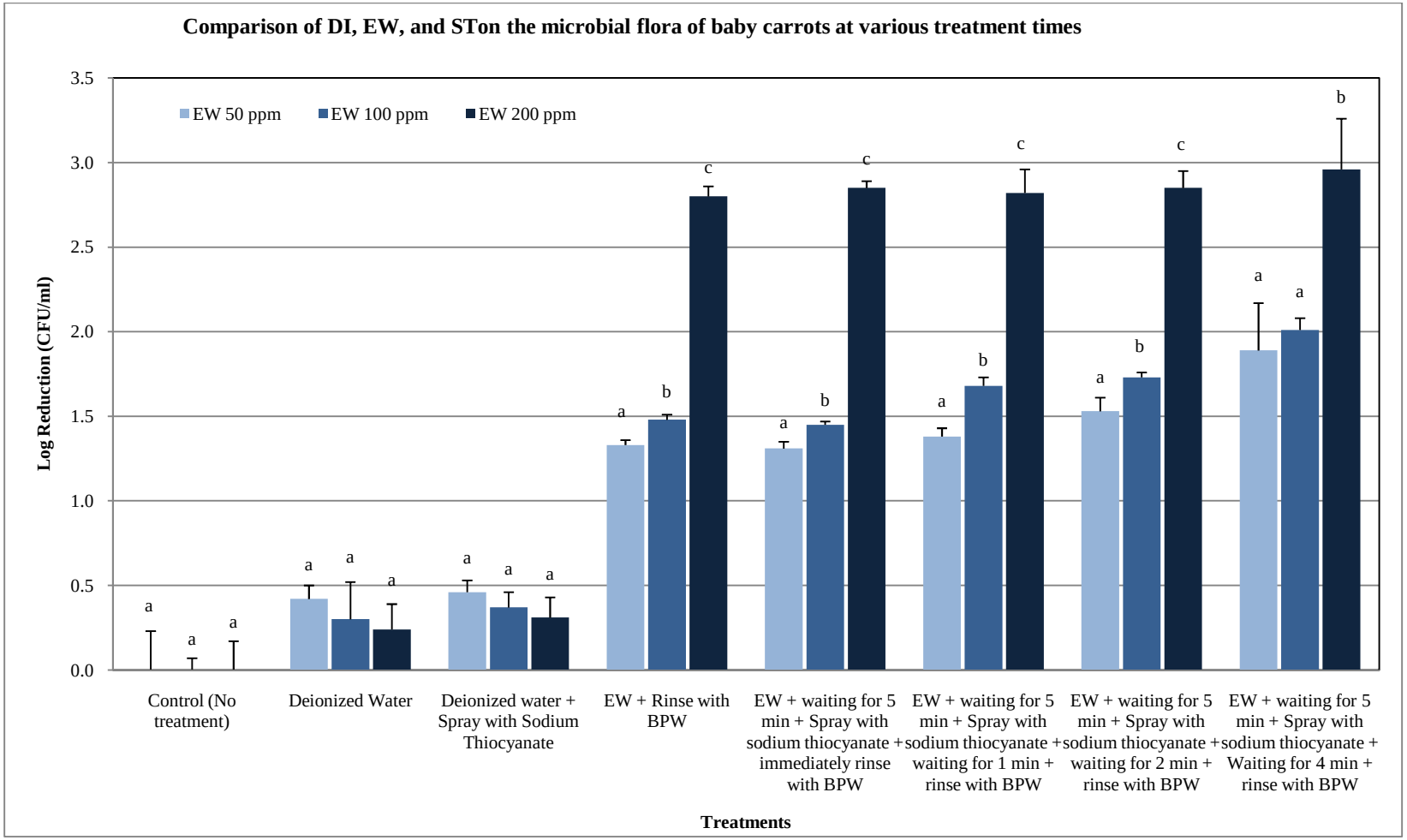


Figure 10. Baby carrots were immersed in electrolyzed water (50, 100, or 200 ppm) for 1 min followed by spraying with 100 mM sodium thiocyanate for 30 sec. Log reduction of indigenous bacteria on baby carrots after washing with the electrolyzed water for 1, 2, or 4 min, allowed to drain for 5 min then spray processed with sodium thiocyanante. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same treatment.

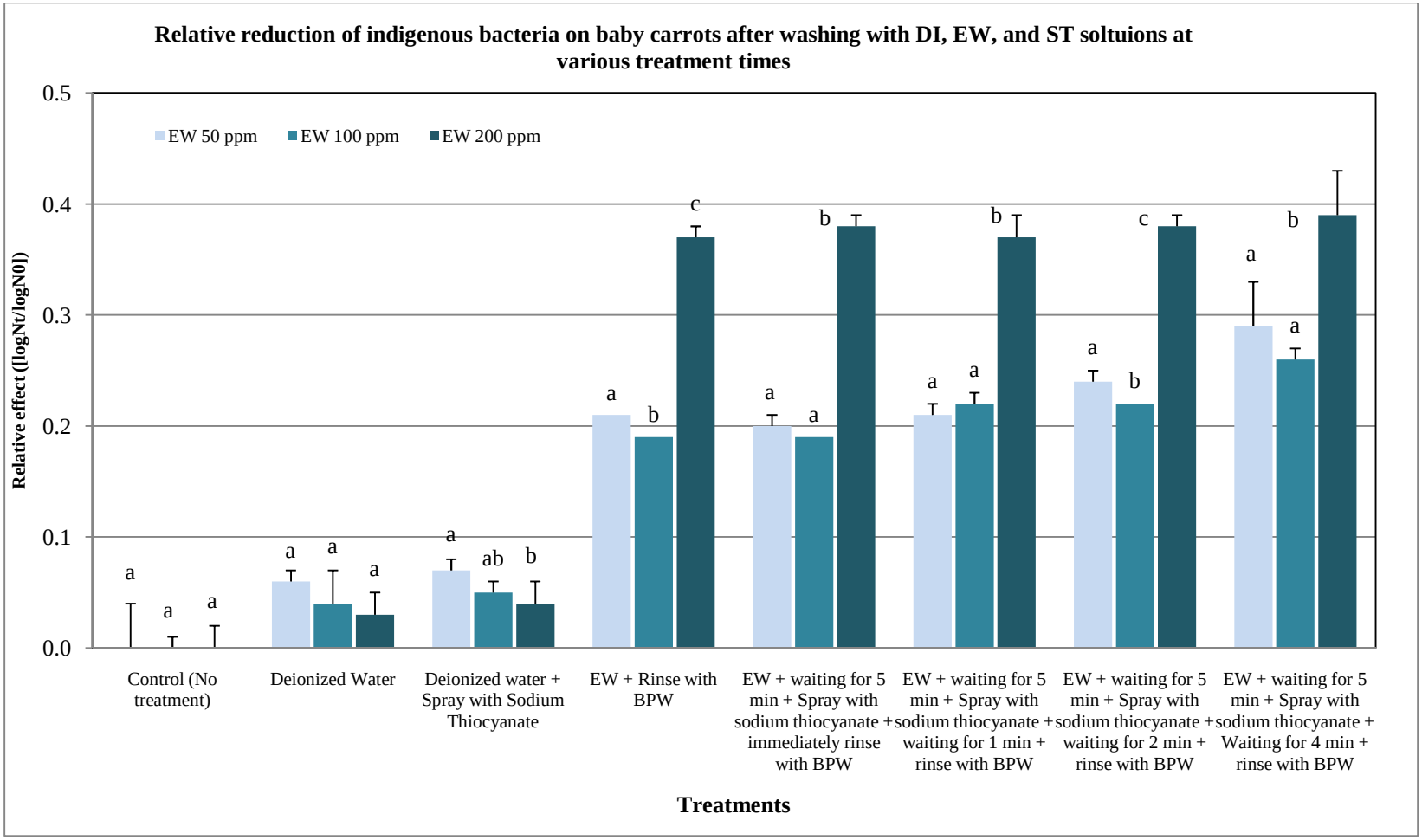


Figure 11. Baby carrots were immersed in electrolyzed water (50, 100, or 200 ppm) for 1 min followed by spraying with 100 mM sodium thiocyanate for 30 sec. Relative reduction of bacteria on baby carrots after washing with the electrolyzed water for 1, 2, or 4 min, allowed to drain for 5 min then spray processed with sodium thiocyanante. The data points represent the means of multiple replications and error bars represent standard deviation from the mean. Treatments with different lower case letters are significantly different ($P < 0.05$) for the same treatment.

REFERENCES

Abadias, M., J. Usall, M. Oliveira, I. Alegre, and I. Vinas. 2008. Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. Food Microbiol. 123: 151-158.

Al-Ghazali, M. R., and S. K. Al-Azawi. 1990. *Listeria monocytogenes* contamination of crops grown on soil treated with sewage sludge cake. J. Appl. Bacteriol. 69: 642-647.

Ayebah, B., Y. C. Hung, and J. F. Frank. 2006. Enhancing the bactericidal effect of electrolyzed water on *Listeria monocytogenes* biofilms formed on stainless steel. J. Food Prot. 68: 1375-1380.

Bailey, J. S., J. A. Cason, and N. A. Cox. 1998. Effect of *Salmonella* in young chicks on competitive exclusion treatment. Poultry Sci. 77: 394-399.

Bari, M. L., Y. Sabina, S. Isobe, T. Uemura, and K. Isshiki. 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monobytogenes* on the surface of tomatoes. J. Food Protect. 66: 542-548.

Barrette Jr. W. C., D. M. Hannum, W. D. Wheeler, and J. K. Hurst. 1989. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. Biochem. J. 28: 9172-9178.

Bartz, J. A., C. G. Eayre, M. J. Mahovie, D. E. Concelmo, J. K. Brecht, and S. A. Sargent. 2005. Chlorine concentration and the inoculation of tomato fruit in packinghouse dump tanks. Plant Dis. 85: 885-889.

Beczner, J., and I. B. Vidács. 2009. Microbiology of plant foods and related aspects. Acta Aliment Hung. 38: 99-115.

Berger, C. N., S. V. Sodha, R. K. Shaw, P. M. Griffin, D. Pink, P. Hand, and G. Frankel. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. 12: 2385-2397.

Besser, R. E., P. M. Griffin, and S. Laurence. 1990. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: An emerging infectious disease. Annu. Rev. Med. 50: 355-367.

Beuchat, L. R., and R. E. Brackett. 1990a. Inhibitory effects of raw carrots on *Listeria monocytogenes*. Appl. Environ. Microbiol. 56: 1734-1742.

Beuchat, L. R., and R. E. Brackett. 1990b. Growth of *listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging, temperature and time. J. Food Sci. 55: 755-758.

Beuchat. L. R., and R. E. Brackett. 1991. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. Appl. Environ. Microbiol. 57:1367-1371.

Beuchat, L. R. 1996. *Listeria monocytogenes*: incidence on vegetables. Food Control. 7: 223-228.

Beuchat L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. J. Food Prot. 62: 845-849.

Bower, C. K., and M. A. Daeschel. 1999. Resistance responses of microorganisms in food environments. Int. J. Food Microbiol. 50: 33-44.

Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Post Bio. Technol. 15: 305-311.

Brecht, J. K. 1995. Physiology of lightly processed fruits and vegetables. Horticulture Sci. 301: 9-22.

Brown, J., T. Ross, P. D. Nichols, and T. A. McMeekin. 1997. Acid habituation of *Escherichia coli* and the potential role of cyclopropane and fatty acids in low pH tolerance. Int. J. Food Microbiol. 37: 163-173.

Burnett, S. L., and L. R. Beuchat. 2001. Food-borne pathogens: Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. J. Ind. Microbiol. Biot. 27: 104-110.

CDC. 1993. Multi-state outbreak of *Salmonella* serotype Montevideo infection. EPI-AID, 93-97. Atlanta, GA.

CDC. 2007. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants – United States, 2005-2006. MMWR weekly. 56: 909-911.

Chavant, P., B. Martinie, T. Meylheuc, and M. Hebraud. 2002. *Listeria monocytogenes* LO28: Surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. Appl. Environ. Microbiol. 68: 728-737.

Cherry. J. P. 1999. Improving the safety of fresh produce with antimicrobials. Food Technol. 53: 54-59.

Cox, N. A., J. S. Bailey, J. M. Mauldin, and L. C. Blankenship. 1990. Research note: Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poultry Sci. 69: 1606-1609.

Crutchfield, S. R. and T. Roberts. 2000. Food safety efforts accelerate in the 1990s. Food Res. 23: 44-49.

Davidon, P. M. and M. A. Harrison. 2002. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. Food Technol. 56: 69-78.

Deza, M. A., M. Araujo, and M. J. Garrido. 2003. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. Lett. Appl. Microbiol. 37: 482-487.

Doyle, M. P. 1990. Fruit and vegetable safety-microbiological considerations. Int. J. Food Sci. 34: 1-22.

Farber, J. M. and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Mol Biol R. 55(3): 476-511.

Farrel, B. L., A. B. Ronner, and A. C. Lee Wong. 1998. Attachment of *Escherichia coli* O157:H7 in ground beef to meat grinders and survival after sanitation with chlorine and peroxyacetic acid. J. Food Prot. 61: 817-822.

FDA. 1998. Guidance for industry: Guide to minimize microbial food safety hazards for fresh fruits and vegetables. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM064574. Accessed Feb.25, 2008.

FDA. 2000. Sdoium diacetate, sodium acetate, sodium lactate and potassium lactate; Use as food additives. Fed. Reg. 65: 17128-17129. Washington, D. C. Accessed Jan. 20, 2000.

Francis, G. A., T. Christopher, and D. O'Beirne. 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Tech. 34: 1-12.

Garcia-Villanova Ruiz, B., R. Galvez Vargas, and R. Garcia-Villanova. 1987. Contamination on fresh vegetables during cultivation and marketing. Int. J. Food. Microbiol. 4: 285-291.

Grant, J., A. M. Wendelboe, A. Wendel, B. Jepson, P. Torres, C. Smelser, and R. T. Rolfs. 2006. Spinach-associated *Escherichia coli* O157:H7 outbreak, Utah and New Mexico, 2006. http://www.cdc.gov/EID/content/14/10/1633.htm Accessed Oct. 2008.

Guentzel, J. L., K. L .Lam, M. A. Callan, S. A. Emmons, and V. L. Dunham. 2008. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. Food Microbiol. 25: 36-41.

Han, Y., D. M. Sherman, R. H. Linton, S. S. Nielsen, and P. E. Nelson. 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. Food Microbiol. 17: 521-533.

Heisick, J. E., D. E. Wagner, M. L. Niermand, and J. T. Peeler. 1989. *Listeria spp*. found on fresh market produce. Appl. Environ. Microbiol. 55: 1925-1927.

Hilgren, J. D. and J. A. Salverda. 2006. Antimicrobial efficacy of a peroxyacetic/octanoic acid mixture in fresh-cut vegetable process waters. J. Food Sci. 65: 1376-1379.

Hirano, H. and O.Ueda. 1997. Characteristics of electrolyzed neutral water possibility of the practical use for food hygiene. Shokuhin Kogyo. 40: 25-35.

Ho, J. L., K. N. Shands, G. Freidland, P. Eckind, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. 146: 520-524.

Hricova, D., R. Stephan, and C. Zweifei. 2008. Electrolyzed water and its application in the food industry. J. Food Prot. 71: 1934-1947.

Hua, G. H. and D. A. Reckhow. 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. Water Res. 41: 1667-1678.

Huang, Y. R., H. S. Hsieh, S. Y. Lin, S. J. Lin, Y. C. Hung, and D. F. Hwang. 2006. Application of electrolyzed oxidizing water on the reduction of bacterial contamination for seafood. Food Control. 17: 987-993.

Huang, Y. R., Y. C. Hung, S. Y. Hsu, Y. W. Huang, and D. F. Hwang. 2008. Application of electrolyzed water in the food industry. Food Control. 19: 329-345.

Hurst, J. K., W. C. Brewer Jr, B. R. Michel, and H. Rosen. 1991. Hypochlorous acid and myeloperoxidase-catalyzed oxidation of iron-sulfur clusters in bacterial respiratory dehydrogenase. Eur. J. Biochem. 202: 1275-1282.

Hyun. G. Y., J. A. Bartz, and K. R. Schneider. 2005. Effectiveness of individual or combined sanitizer treatments for inactivating *Salmonella spp*. on smooth surface, stem scar, and wounds of tomatoes. Food Microbiol Safety. 70: 409-414.

Jay, J. M. 1970. Modern Food Microbiology. Press, Van Nostrand Reinhold, New York.

Katta, S. R., D. R. Rao, R. Dunki, and C. B. Chawan. 1991. Effect of gamma irradiation of whole chicken carcasses on bacterial loads and fatty acids. J. Food Sci. 56: 371-373.

Keskinen, L. A., A. Burke, and B. A. Annous. 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:h7 from lettuce leaves. Int. J. Food Microbial. 132: 134-140.

Kim, C., Y. C. Hung, and R. E. Brackett. 2000a. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. Int. J. Food Microbiol. 61: 199-207.

Kim, C., Y. C. Hung, and R. E. Brackett. 2000b. Rules of oxidation reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. J. Food Prot. 63: 19-24.

Kim. C., Y. C. Hung, R. E. Brackett, and J. F. Frank. 2001. Inactivation of *listeria monocytogenes* biofilms by electrolyzed oxidizing water. J. Food Sci. 25: 91-100.

Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. J. Food Prot. 64: 652-658.

Koseki, S., K. Fujiwara, and K. Itoh. 2002. Decontaminative effect of frozen acidic electrolyzed water on lettuce. J. Food Prot. 65: 411-414.

Koseki, S., K. Yoshida, Y. Kamitani, S. Isobe, and K. Itoh. 2004a. Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157:H7 and *Salmonella* on lettuce. Food Microbiol. 21: 559-566.

Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2004b. Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. J. Food Prot. 67: 1247-1251.

Kumar, C. G., and S. K. Anand. 1998. Significance of microbial biofilms in food industry: a review. Int. J. Food Microbiol. 42: 9-27.

Leyer, G. J., and E. A. Johnson. 1997. Acid adaptation sensitizes *Salmonella typhimurium* to hypochlorous acid. Appl. Environ. Microbiol. 63: 461-467.

Lin. C. M. and C. I. Wei. 1997. Transfer of *Salmonella Montevideo* onto the interior surfaces of tomatoes by cutting. J. Food Prot. 60: 858-863.

Lin, C. M., S. S. Moon., M. P. Doyle, and K. H. McWatters. 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteric Serotype Enteritidis*, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. J. Food Prot. 65: 1215-1220.

Lin, C. S., C. Wu., J. Y. Yeh, and R. K. Saalia. 2005. The evaluation of electrolysed water as an agent for reducing micro-organisms n vegetables. Int. J. Food Sci. Tech. 40: 495-500.

Liston, J. 1990. Microbial hazards of seafood consumption. Food Technol. 44: 56-62.

Lillard, H. S, and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bactericide in poultry chiller water. J. Food Sci. 48: 125-126.

Mahmoud, B. S. M., K. yamazaki, K. Miyashita, I. S. Shin, C. Dong-suk, and T. Suzuki. 2004. Decontamination effect of electrolyzed NaCl solutions on carp. Lett Appl Microbiol. 39: 169-173.

Mahound, B. S. M., K. Yamazaki., K. Miyashita., S. II-Shik, C. Dong-Suk, and T. Suzuki. 2004. Decontamination effect of electrolyzed NaCl solutions on carp. Lett Appl Microbiol. 39: 169-173.

Mahmoud, B. S. S., Y, Kawai, K, Yamazaki, K, Miyashita, H, Shin, and T, Suzuki. 2006. A new technology for fish preservation by combined treatment with electrolyzed NaCl solutions and essential oil compounds. Food Chem. 99: 656-662.

Maria, E. G., G. Andrea, R. C. Maria, and S. Milena. 1996. Shelf-life modeling for fresh-cut vegetables. Postharvest Biol. Technol. 9: 195-207.

McMeekin, T. A., J. Brown, K. Krist, D. Miles, K. Neumeyer, D. S. Nichols. J. Olley, K. Presser, D. A. Ratkowsky, T. Ross, M. Salter, and S. Soontranon. 1997. Quantitative microbiology: A basis for food safety. Emerg Infect Dis. 3: 541-549.

Mead, P. S. and P. M. Griffin. 1998. *Escherichia coli* O157:H7. Lancet 352: 1207-1212.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, and C. Shapiro. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5: 607-625.

Molenda, J. R., W. G. Johnson, M. Gishbein, B. Wentz, I. J. Mehlamn, T. A. Jr. Dadisman. 1972. *Vibrio parahaemolyticus* gastroenteritis in Maryland: laboratory aspects. Appl. Microbiol. 24: 444-448.

Nicholson, F. A., S. J. Groves, and B. J. Chambers. 2005. Pathogen survival during livestock manure storage and following land application. Bioresource Technol. 96: 135-143.

Nguyen-the, C. and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. J. Food Sci. 34: 371-401.

Oh, D. H. and D. L. Marshall. 1993. Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against *Listeria monocytogenes*. Int. J. Food Microbiol. 20: 239-246.

Oh, D. H. and D. L. Marshall. 1994. Enhanced inhibition of *Listeria monocytogenes* by glycerol monolaurate with organic acids. J. Food Sci. 59: 1258–1261.

Ölmez, H. 2010. Effect of different sanitizing methods and incubation time and temperature on inactivation of *Escherichia coli* on lettuce. J. Food Safety. 30: 288-299.

Olsen, S. J., L. C. MacKinon., J. S. Goulding, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks-United States, 1993-1997. Morbidity and Mortality Weekly Report 49: 1-51. Atlanta, GA.

Park, C. M., Y. C. Hung, M. P. Doyle, G. O. I. Ezeike, and C. Kim. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. J. Food Sci. 66: 1368-1372.

Park, H., Y. C. Hung, and R. E. Brackett. 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. Int. J. Food Microbiol. 72: 77-83.

Park, E. J., E. Alexander., G. A. Taylor., R. Costa, and D. H. Kang. 2008. Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. Food Microbiol. Safety. 73: 268-272.

Park, C. M., Y. C. Hung, and R. E. Brackett. 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. Int. J. Food Microbiol. 72: 77-83.

Park, H., Y. C. Hung, and D. H. Chung. 2004. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Food Microbiol. 91: 13-18.

Park, E. J., E. Alexander, G. A. Taylor, R. Costa, and D. H. Kang. 2008. Effect of electrolyzed water for reduction of foodborne pathognes on lettuce and spinach. J. Food Sci. 73: 268-272.

Pimentel, D., S. McNAir, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal and microbe invasions. Agric. Ecosyst. Environ. 84: 1-20.

Rahman, S. M. E., D. T, and H. O. Deog. 2010. Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. Food Control. 21: 1383-1387.

Ramaswamy, V., V. M. Cresence, J. S. Rejitha, M. U. Lekshmi, K. S. Dharsana, S. P. Prasad, and H. M. Vijila. 2007. Listeria-review of epidemiology and pathogenesis. J. Microbiol Immun Infect. 40: 4-13.

Rangel, J. M., P. H. Sparling, C. Collen, P. M. Griffin, and D. L. Swerdlow. Epidemiology of Escherichia coli O157:H7 outbreaks, United States. 2005. http://www.cdc.gov/eid. Accessed Apr. 2005.

Rico, D., A. B. M. Diana, J. M. Barat, and C. B. Ryan. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Food Sci. Technol. 18: 373-386.

Roberts, T. A., R. B. Tompkin, A. C. Baird-Parker, and F. International Commission on Microbiological Specifications (ICMSF). 1996. Microorganisms in foods. 5: Microbiological specifications of food pathogens. Chapman and Hall. London.

Roever, C. D. 1998. Microbiological safety evaluations and recommendations on fresh produce. Food Control. 9: 321-347.

Powell, S. C., and R. W. Attwell. 2000. The use of epidemiological data to direct resources in food safety control. Res. Environ Health. 15: 381-387.

Ruiz. B. G. V., R. G. Vargas, and R. G. Villanova. 1987. Contamination on fresh vegetables during cultivation and marketing. Int. J. Food Microbiol. 4: 285-291.

Russell, A. D. 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. J. Appl. Bacterial. 71: 191-201.

Russell, S. M. 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on surface of eggs. Poultry. Sci. 82: 158-162.

Ryan, K. R., and C. G. Ray. 2004. Medical microbiology: An introduction to infectious diseases. 4th ed. McGraw-Hill.

Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, W. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nichols, and C. V. Broome. 1983. Epidemic listeriosis- Evidence for transmission by food. N. Engl. J. Med. 308: 203-206.

Schuchat, A., B. Swaminathan, and C. V. Broome. 1991. Epidemiology of human Listeriosis. Clinical. Microbiol. Res. 4: 169-183.

Shank, F. R., E. L. Elliot, I. K. Wachsmuth, and M .E. Losikoff. 1996. US position on *Listeria monocytogenes* in foods. Food Control. 7: 229-234.

Shen, Y. L., Y. Liu, Y. F. Zhang, J. Cripe, W. Conway, J. H. Meng, G. Hall, and A. A. Bhagwat. 2006. Isolation and characterization of *listeria monocytogenes* isolates from ready-to-eat foods in Florida. Appl. Environ. Microbiol. 72(7): 5073-5076.

Singh, N., R. K. Singh, A. K. Bhunia, and R. L. Stroshine. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. Lebensm. Wiss. Technol. 35: 720-729.

Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67: 2342-2353.

Sizmur, K. and C. W. Walker. 1998. *Listeria* in prepacked salads. Lancet 1: 8985-1167

Steele, M., and J. Odumeru. 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. J. Food Prot. 67: 2839-2849.

Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. Microbial hazards and emerging issues associated with produce. A preliminary report to the national advisory committee on microbiological criteria for foods. J. Food Prot. 60: 1400-1408.

Udompijitkul, P., M. A. Daeschel, and Y. Zhao. 2007. Antimicrobial effect of electrolyzed oxidizing water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh strawberries. J. Food Sci. 72: 397-406.

Venkitanarayanan, K. S., G. O. Ezeike, Y. C. Hung, and M. P. Doyle. 1999. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. Appl. Environ. Microbiol. 65: 4276-4279.

Vivien, M. A., E. L. Janet, H. C. Burton, R. T. Whyte, and G. C. Mead. 2000. Hygiene aspects of modern poultry chilling. Int. J. Food Microbiol. 58: 39-48.

Wang, H., H. Feng, and Y. G. Luo. 2006. Dual-phasic inactivation of *Escherichia coli* O157:H7 with peroxyacetic acid, acidic electrolyzed water and chlorine on cantaloupes and fresh-cut apples. J. Food Safety. 26: 335-347.

Wei, C. I., T. S. Huang, J. M. King, W. F. Lin, M. L. Tamplin, and J. A. Bartz. 1995. Growth and survival of *Salmonella Montevideo* on tomatoes and disinfection with chlorinated water. J. Food Prot. 58: 829-836.

Welshimer, H. J. 1960. Survival of *Listeria monocytogenes* in the soil. J. Bacteriol. 80: 316-320.

Welshimer, H. J. 1968. Isolation of *Listeria monocytogenes* from vegetation. J. Bacteriol. 95: 300-303.

White, P. L., A. R. Baker, and W. O. James. 1997. Strategies to control *Salmonella* and *Campylobacter* in raw poultry products. Res. Sci. Technol. Int. Epiz. 16: 525-541.

Wilcox, F., P. Tobback, and M. Hendrickx. 1994. Microbial safety assurance of minimally processed vegetables by implementation of the Hazard Analysis Critical Control Point System. Acta Aliment Hung. 23: 221-238.

Wisniewsky, M. A., B. A. Glatz, M. L. Gleason, and C. A. Reitmeier. 2000. Reduction of *Escherichia coli O157:H7* counts on whole fresh apples by treatment with sanitizers. J. Food Prot. 63: 703-708.

Wright, J. R., S. S. Sumner, C. R. Hackney, M. D. Pierson, and B. W. Zoecklein. 2000. Food and Environmental Sanitation. Dairy Food Environ. 20: 120-126.

Wood, R. C., C. Hedberg, and K. White. 1991. A multistate outbreak of *Salmonella javiana* associated with raw tomatoes, p. 69. Abst., Epidemic Intelligence Service 40th Annu. Conf., CDC, Altlanta, GA. (Abstr.)

Woolthuis, C. H. J., and F. J. M. Smulders. 1085. Microbial decontamination of calf carcasses by lactic acid sprays. J. Food Prot. 48: 832-837.

Wu, C.H., and B. Kim. 2007. Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. Food Microbiol. 24: 794-800.

Zeitoun, A. A. M., and J. M. Debevere. 1990. The effect of treatment with buffered lactic acid on microbial decontamination and on shelf life of poultry. Int. J. Food Microbiol. 11: 305-312.

Zhang, S., and J. M. Farber. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh cut vegetables. Food Microbiol. 13: 311-321.

Zhang G. D., M. L. Ma, V. H. Phelan, and M. P. Doyle. 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. J. Food Prot. 72: 1392-1397.

APPENDIX

The following Tables (chemical analysis of liquid treatments) correlate directly to the Figure graphs in the thesis with the same Figure number.

For the EW treatment, the products, treatment methods, weight of products, the contact surface area of each sample, dipping time, FAC levels of EW before and after treatment, pH and ORP of the various chemical solutions are identified before and after treatment.

For the LA and PAA treatments, the products, treatment methods, weight of products, the contact surface area of each sample, dipping time, pH and ORP of the various chemical solutions are identified before and after treatment.

For the LA combined with EW treatment, the products, treatment methods, weight of products, the contact surface area of each sample, dipping time, FAC levels of the combined solution before and after treatment, pH and ORP of the combined solutions are identified before and after treatment.

Table 1A. Chemical analysis of sanitizing solutions for 1-min EW treatment of carrots (Fig. 1)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area,cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			49.53	150.26							
Ctrl2			50.21	156.92							
Ctrl3			46.5	154.33							
Ctrl4			59.02	148.26							
Water1	Dip		60.1	149.26	1			6.71	6.71	471	470
Water2	Dip		52.32	148.52	1			6.75	6.74	474	473
Water3	Dip		54.62	149.1	1			6.75	6.73	470	471
Water4	Dip		59.1	162.07	1			6.73	6.73	475	474
Carrot1-50	Dip	1	70.2	156.2	1	53	46	6.44	6.42	851	850
Carrot2-50	Dip	1	54.26	152.21	1	52	48	6.51	6.49	851	851
Carrot3-50	Dip	1	56.39	153.06	1	49	44	6.41	6.38	850	850
Carrot4-50	Dip	1	50.21	150.44	1	50	41	6.5	6.45	850	848
Carrot1-100	Dip	1	46.2	156.24	1	106	85	6.38	6.33	862	861
Carrot2-100	Dip	1	48.25	152.4	1	105	79	6.35	6.3	868	865
Carrot3-100	Dip	1	49.6	150.62	1	102	88	6.34	6.3	868	867
Carrot4-100	Dip	1	57.13	149.8	1	107	89	6.35	6.3	870	867
Carrot1-200	Dip	1	56.03	156.21	1	208	156	6.12	6.08	921	920
Carrot2-200	Dip	1	51.7	152.21	1	195	159	6.15	6.11	923	921
Carrot3-200	Dip	1	59.2	148.26	1	198	146	6.14	6.1	922	920
Carrot4-200	Dip	1	51.12	146.52	1	186	171	6.18	6.1	923	920
Note: Initial solution levels: EW (218 ppm), ORP (896 mV), pH (6.14)											

Table 1B. Chemical analysis of sanitizing solutions for 1-min EW treatment of grape tomatoes (Fig. 1)

Product	Treatment method	EW volume (L)	Product (gms)	Product (surface area, cm^2)	Treatment time (min)	PPM Cl- (start)	PPM Cl- (finish)	pH (start)	pH (end)	ORP (start)	ORP (end)
Ctrl1			60.79								
Ctrl2			58.76								
Ctrl3			64.27								
Ctrl4			55.62								
Water1	Dip		71.66	160.855	1			7.03	7.02	543	543
Water2	Dip		69.27	154.2652	1			7.06	7.06	541	540
Water3	Dip		62.3	149.4479	1			7.04	7.04	543	541
Water4	Dip		65.47	150.2882	1			7.04	7.03	543	543
Tomato1-50	Dip	1	73.72	141.3765	1	46	24	7.18	7.15	840	839
Tomato2-50	Dip	1	62.6	150.8016	1	48	26	7.12	7.11	838	837
Tomato3-50	Dip	1	68.15	147.2258	1	50	23	7.15	7.13	841	840
Tomato4-50	Dip	1	71.69	154.8002	1	50	21	7.17	7.14	840	840
Tomato1-100	Dip	1	74.05	159.2212	1	98	72	6.7	6.68	911	907
Tomato2-100	Dip	1	62.35	150.8016	1	100	65	6.7	6.68	910	908
Tomato3-100	Dip	1	65.7	155.4026	1	101	61	6.7	6.65	913	910
Tomato4-100	Dip	1	73.12	145.6613	1	102	75	6.68	6.64	911	909
Tomato1-200	Dip	1	59.63	136.0984	1	196	163	6.2	6.1	962	960
Tomato2-200	Dip	1	64.87	150.6954	1	198	167	6.25	6.2	958	957
Tomato3-200	Dip	1	68.25	148.2232	1	202	152	6.23	6.18	959	957
Tomato4-200	Dip	1	62.56	147.981	1	200	147	6.2	6.19	961	959

Note: Initial solution levels: EW (276 ppm); ORP (821 mV); pH (6.38).

Table 1C. Chemical analysis of sanitizing solutions for 1-min EW treatment of cabbage (Fig. 1)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			21.3	152.3							
Ctrl2			24.45	157							
Ctrl3			19.87	161.1							
Ctrl4			16.2	160							
Water1	Dip		20.88	169	1			7.02	7.01	521	520
Water2	Dip		19.3	157.2	1			7.02	7.01	523	521
Water3	Dip		16.65	155	1			7.02	7.01	523	524
Water4	Dip		17.2	147.5	1			7.02	7.02	522	521
Cabbage1-50	Dip	1	32.51	146	1	52	24	6.76	6.74	825	823
Cabbage2-50	Dip	1	20.34	160.4	1	51	22	6.76	6.75	825	823
Cabbage3-50	Dip	1	12.06	163.2	1	50	21	6.77	6.75	825	824
Cabbage4-50	Dip	1	11.83	156	1	50	27	6.76	6.75	825	823
Cabbage1-100	Dip	1	15.9	143	1	98	67	6.58	6.57	871	870
Cabbage2-100	Dip	1	15.88	148	1	97	65	6.58	6.57	871	870
Cabbage3-100	Dip	1	25.11	156	1	101	70	6.59	6.56	871	869
Cabbage4-100	Dip	1	17.02	151.8	1	100	68	6.58	6.56	871	869
Cabbage1-200	Dip	1	18.6	157	1	200	152	6.55	6.54	898	897
Cabbage2-200	Dip	1	14.68	160	1	200	144	6.55	6.54	897	896
Cabbage3-200	Dip	1	13.23	155	1	203	147	6.55	6.54	898	896
Cabbage4-200	Dip	1	17.2	154.6	1	204	148	6.54	6.52	898	897
Note: Initial solution levels: EW (232 ppm); ORP (841 mV); pH (6.22).											

Table 1D. Chemical analysis of sanitizing solutions for 1-min EW treatment of pepper (Fig. 1)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			36.02	146.55							
Ctrl2			41.21	148.25							
Ctrl3			50.11	153.6							
Ctrl4			39.8	156							
Water1	Dip		35.45	169.65	1			7.09	7.09	479	479
Water2	Dip		36.21	154.21	1			7.1	7.1	479	479
Water3	Dip		47.1	147.6	1			7.1	7	479	478
Water4	Dip		40.8	140.58	1			7.09	7.07	479	478
Pepper1-50	Dip	1	47.81	138.54	1	51	41	6.65	6.63	877	876
Pepper2-50	Dip	1	52.44	144.6	1	51	38	6.65	6.64	875	874
Pepper3-50	Dip	1	56.4	152.4	1	50	37	6.65	6.64	875	874
Pepper4-50	Dip	1	46.21	148.24	1	48	38	6.65	6.64	876	875
Pepper1-100	Dip	1	42.07	162.86	1	104	72	6.59	6.58	898	897
Pepper2-100	Dip	1	36.12	155.75	1	102	67	6.58	6.58	897	897
Pepper3-100	Dip	1	38.22	147.2	1	98	69	6.59	6.57	897	896
Pepper4-100	Dip	1	43.19	150.6	1	97	65	6.59	6.57	898	896
Pepper1-200	Dip	1	34.49	150.8	1	189	165	6.42	6.41	920	917
Pepper2-200	Dip	1	36.25	152.24	1	195	168	6.4	6.4	918	917
Pepper3-200	Dip	1	40.16	147.6	1	194	159	6.42	6.4	918	917
Pepper4-200	Dip	1	39.27	149.25	1	192	162	6.41	6.4	919	917
Note: initial solution levels: EW (218 ppm); ORP (893 mV); pH (6.52).											

Table 1E. Chemical analysis of sanitizing solutions for 1-min EW treatment of spinach (Fig. 1)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			4.84	144							
Ctrl2			5.45	152.5							
Ctrl3			5.89	158							
Ctrl4			6.2	160							
Water1	Dip		8.67	140	1			7.03	6.98	597	596
Water2	Dip		8.27	142.5	1			7.05	7	597	596
Water3	Dip		9.08	156	1			7.03	6.97	596	594
Water4	Dip		8.64	160	1			7.03	6.98	597	596
Spinach1-50	Dip	1	7.41	162	1	46	25	6.83	6.82	809	807
Spinach2-50	Dip	1	8.19	160	1	48	24	6.83	6.82	809	808
Spinach3-50	Dip	1	8.28	157.5	1	48	27	6.83	6.83	809	808
Spinach4-50	Dip	1	7.5	152	1	49	21	6.83	6.8	809	808
Spinach1-100	Dip	1	8.2	150	1	102	65	6.76	6.74	814	813
Spinach2-100	Dip	1	6.18	132	1	104	61	6.75	6.73	815	813
Spinach3-100	Dip	1	6.63	147.5	1	102	67	6.75	6.74	815	813
Spinach4-100	Dip	1	7.12	144	1	105	59	6.75	6.73	814	813
Spinach1-200	Dip	1	5.8	160	1	198	134	6.69	6.67	831	830
Spinach2-200	Dip	1	5.84	144	1	204	141	6.69	6.67	831	830
Spinach3-200	Dip	1	6.25	152.5	1	199	138	6.68	6.67	831	829
Spinach4-200	Dip	1	6.71	156	1	200	135	6.68	6.67	830	828
Note: Initial solution levels: EW (210 ppm); ORP (914 mV); pH (6.35).											

Table 2A. Chemical analysis of sanitizing solutions for 2-min EW treatment of carrots (Fig. 2)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			54.95	152.98							
Ctrl2			52.79	150.55							
Ctrl3			54.4	148.75							
Ctrl4			48.9	146.22							
Water1	Dip		56.6	152.30	2			6.76	6.76	467	466
Water2	Dip		51.8	150.91	2			6.73	6.72	465	465
Water3	Dip		52.24	147.67	2			6.75	6.75	464	464
Water4	Dip		56.71	153.39	2			6.76	6.76	465	464
Carrot1-50	Dip	1	66.92	154.57	2	55	39	6.47	6.46	847	847
Carrot2-50	Dip	1	56.66	152.18	2	47	43	6.55	6.55	845	844
Carrot3-50	Dip	1	62.09	154.32	2	52	41	6.55	6.53	845	845
Carrot4-50	Dip	1	51.66	149.42	2	51	35	6.54	6.52	849	849
Carrot1-100	Dip	1	58.7	146.91	2	100	81	6.45	6.42	855	855
Carrot2-100	Dip	1	57.75	150.42	2	106	74	6.45	6.43	855	855
Carrot3-100	Dip	1	50.27	150.42	2	100	82	6.47	6.44	856	856
Carrot4-100	Dip	1	58.84	148.23	2	98	83	6.45	6.42	864	863
Carrot1-200	Dip	1	54.1	153.96	2	204	133	6.17	6.12	934	934
Carrot2-200	Dip	1	52.86	151.68	2	198	145	6.18	6.13	907	905
Carrot3-200	Dip	1	49.5	147.16	2	200	124	6.19	6.15	909	908
Carrot4-200	Dip	1	54.62	152.97	2	199	151	6.19	6.15	905	905
Note: Initial solution levels: EW (221 ppm), ORP (876 mV), pH (6.23)											

Table 2B. Chemical analysis of sanitizing solutions for 2-min EW treatment of grape tomatoes (Fig. 2)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			60.79	145.27							
Ctrl2			58.76	147.86							
Ctrl3			64.27	153.65							
Ctrl4			55.62	148.98							
Water1	Dip		56.54	145.27	2			7.03	7.02	543	542
Water2	Dip		66.44	152.35	2			7.06	7.04	541	540
Water3	Dip		62.2	155.74	2			7.04	7.03	542	541
Water4	Dip		59.31	146.77	2			7.04	7.03	543	540
Tomato1-50	Dip	1	65.98	150.42	2	50	19	7.18	7.14	840	840
Tomato2-50	Dip	1	52.34	136.10	2	52	25	7.17	7.12	839	837
Tomato3-50	Dip	1	60.22	149.27	2	50	20	7.12	7.09	837	836
Tomato4-50	Dip	1	70.58	152.98	2	51	17	7.15	7.1	837	835
Tomato1-100	Dip	1	66.32	137.99	2	101	65	6.7	6.68	908	907
Tomato2-100	Dip	1	79.59	156.96	2	95	34	6.65	6.68	912	910
Tomato3-100	Dip	1	58.2	151.66	2	98	51	6.7	6.65	911	910
Tomato4-100	Dip	1	70.23	146.79	2	98	57	6.69	6.64	911	909
Tomato1-200	Dip	1	70.33	150.80	2	194	118	6.4	6.2	959	958
Tomato2-200	Dip	1	76.2	149.80	2	198	102	6.4	6	958	956
Tomato3-200	Dip	1	65.24	154.26	2	198	95	6.3	6.17	958	957
Tomato4-200	Dip	1	73.25	151.28	2	204	123	6.1	6.04	959	958
Note: Initial solution levels: EW (252 ppm), ORP (848 mV), pH (6.21).											

Table 2C. Chemical analysis of sanitizing solutions for 2-min EW treatment of cabbage (Fig. 2)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			21.3	152.3							
Ctrl2			24.45	157							
Ctrl3			19.87	161.1							
Ctrl4			16.2	160							
Water1	Dip		26.88	182.8	2			7.02	7.01	545	543
Water2	Dip		17.69	155	2			7.02	7.01	546	545
Water3	Dip		12.31	154.4	2			7.02	7.01	549	547
Water4	Dip		16.6	150.7	2			7.02	7.02	545	545
Cabbage1-50	Dip	1	25.61	137.5	2	46	19	6.76	6.73	825	823
Cabbage2-50	Dip	1	12.42	152	2	46	17	6.76	6.74	825	823
Cabbage3-50	Dip	1	18.65	168	2	46	17	6.77	6.75	825	823
Cabbage4-50	Dip	1	20.11	152.7	2	48	20	6.76	6.74	825	822
Cabbage1-100	Dip	1	19.63	162	2	92	53	6.58	6.56	871	869
Cabbage2-100	Dip	1	27.06	160	2	95	42	6.58	6.56	871	869
Cabbage3-100	Dip	1	20.88	144	2	94	41	6.59	6.55	871	868
Cabbage4-100	Dip	1	15.56	151.3	2	94	48	6.58	6.56	871	869
Cabbage1-200	Dip	1	17.74	175	2	185	112	6.55	6.53	898	896
Cabbage2-200	Dip	1	27.96	170	2	189	118	6.55	6.54	897	896
Cabbage3-200	Dip	1	24.34	168	2	187	123	6.55	6.53	898	897
Cabbage4-200	Dip	1	19.22	156	2	184	120	6.54	6.53	898	896
Note: Initial solution levels: EW (241 ppm), ORP (905 mV), pH (6.37)											

Table 2D. Chemical analysis of sanitizing solutions for 2-min EW treatment of pepper (Fig. 2)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			23.34	167.76							
Ctrl2			35.5	162.41							
Ctrl3			37.27	158							
Ctrl4			41.22	146.65							
Water1	Dip		44.34	148.79	2			7.09	7.09	479	479
Water2	Dip		46.72	150.32	2			7.1	7	478	477
Water3	Dip		48.58	156.44	2			7.1	7.08	478	478
Water4	Dip		38.65	148.26	2			7.09	7.05	478	477
Pepper1-50	Dip	1	30.93	135.21	2	46	38	6.65	6.62	879	877
Pepper2-50	Dip	1	41.22	142.53	2	51	36	6.64	6.63	876	875
Pepper3-50	Dip	1	43.58	152.3	2	51	32	6.64	6.63	876	875
Pepper4-50	Dip	1	49.62	156.7	2	48	34	6.64	6.63	877	875
Pepper1-100	Dip	1	36.12	158.34	2	95	56	6.6	6.59	910	898
Pepper2-100	Dip	1	38.77	155.21	2	103	58	6.6	6.6	898	897
Pepper3-100	Dip	1	40.12	148.6	2	94	62	6.61	6.59	898	896
Pepper4-100	Dip	1	41.57	146.25	2	89	54	6.6	6.57	899	897
Pepper1-200	Dip	1	44.94	138.33	2	172	142	6.45	6.43	923	921
Pepper2-200	Dip	1	47.2	145.65	2	165	137	6.45	6.43	920	919
Pepper3-200	Dip	1	50.02	152.75	2	170	139	6.43	6.41	920	918
Pepper4-200	Dip	1	45.26	153.6	2	175	136	6.45	6.43	921	918
Note: Initial solution levels: EW (189 ppm); ORP (853 mV); pH (6.46).											

Table 2E. Chemical analysis of sanitizing solutions for 2-min EW treatment of spinach (Fig. 2)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			4.84	144							
Ctrl2			5.45	152.5							
Ctrl3			5.89	158							
Ctrl4			6.2	160.75							
Water1	Dip		4.55	150.21	2			7.03	6.98	597	596
Water2	Dip		4.24	154.5	2			7.05	7	597	595
Water3	Dip		5.87	160.75	2			7.03	6.97	596	595
Water4	Dip		5.28	146	2			7.03	6.98	597	595
Spinach1-50	Dip	1	4.64	150.21	2	54	21	6.83	6.81	809	807
Spinach2-50	Dip	1	6.88	140.04	2	52	20	6.83	6.81	809	808
Spinach3-50	Dip	1	6.62	148.7	2	51	24	6.82	6.81	808	808
Spinach4-50	Dip	1	6.5	144.5	2	49	20	6.83	6.79	809	807
Spinach1-100	Dip	1	5.84	140.04	2	91	61	6.78	6.72	814	812
Spinach2-100	Dip	1	5.09	162	2	95	58	6.78	6.7	815	814
Spinach3-100	Dip	1	6.21	150.21	2	96	54	6.78	6.72	814	813
Spinach4-100	Dip	1	6.47	150.65	2	96	57	6.76	6.72	813	812
Spinach1-200	Dip	1	7.86	160.75	2	102	127	6.7	6.65	830	828
Spinach2-200	Dip	1	4.28	150.21	2	104	138	6.7	6.65	829	828
Spinach3-200	Dip	1	5.51	152.5	2	105	135	6.69	6.65	830	827
Spinach4-200	Dip	1	5.97	148.7	2	102	129	6.68	6.68	830	828
Note: Initial solution levels: EW (223 ppm); ORP (876 mV); pH (6.28).											

Table 3A. Chemical analysis of sanitizing solutions for 4-min EW treatment of carrot (Fig. 3)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			70.12	148.72							
Ctrl2			55.75	157.71							
Ctrl3			65.84	146.15							
Ctrl4			64.49	143.70							
Water1	Dip		66.55	150.67	4			7.14	7.13	486	486
Water2	Dip		69.68	143.38	4			7.23	7.23	487	486
Water3	Dip		64.05	151.30	4			7.26	7.25	486	485
Water4	Dip		76.44	153.81	4			7.25	7.23	485	485
Carrot1-50	Dip	1	88.1	149.16	4	48	29	6.76	7.75	844	843
Carrot2-50	Dip	1	77.26	150.2	4	52	31	6.83	6.8	841	841
Carrot3-50	Dip	1	79.57	149.85	4	52	27	6.85	6.85	842	842
Carrot4-50	Dip	1	70.77	152.93	4	51	25	6.82	6.8	842	840
Carrot1-100	Dip	1	79.07	155.51	4	102	63	6.65	6.62	856	852
Carrot2-100	Dip	1	67.02	151.49	4	101	66	6.74	6.7	846	855
Carrot3-100	Dip	1	62.38	150.79	4	100	65	6.75	6.73	851	848
Carrot4-100	Dip	1	65.21	146.87	4	103	54	6.74	6.69	859	857
Carrot1-200	Dip	1	79.3	146.87	4	195	151	6.55	6.49	903	898
Carrot2-200	Dip	1	72.38	152.41	4	198	147	6.62	6.58	896	894
Carrot3-200	Dip	1	71.72	145.90	4	197	156	6.63	6.6	896	894
Carrot4-200	Dip	1	58.71	145.52	4	201	139	6.62	6.57	898	893
Note: Initial solution levels: EW (284 ppm); ORP (964 mV); pH (6.28)											

Table 3B. Chemical analysis of sanitizing solutions for 4-min EW treatment of grape tomatoes (Fig. 3)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			60.79	145.27							
Ctrl2			58.76	147.86							
Ctrl3			64.27	153.65							
Ctrl4			55.62	148.98							
Water1	Dip		71.66	160.86	4			7.04	7.02	540	538
Water2	Dip		69.27	154.27	4			7.05	7.03	539	537
Water3	Dip		62.3	149.45	4			7.05	7.04	535	534
Water4	Dip		65.47	150.29	4			7.04	7.02	536	536
Tomato1-50	Dip	1	73.23	156.96	4	50	21	7.16	7.13	836	836
Tomato2-50	Dip	1	60.84	145.27	4	52	19	7.12	7.09	837	836
Tomato3-50	Dip	1	57.6	145.31	4	50	15	7.14	7.12	838	838
Tomato4-50	Dip	1	63.3	154.22	4	51	13	7.14	7.11	839	838
Tomato1-100	Dip	1	77.93	159.22	4	99	54	6.68	6.6	910	908
Tomato2-100	Dip	1	64.03	143.66	4	101	60	6.68	6.62	909	908
Tomato3-100	Dip	1	62.1	150.55	4	103	55	6.7	6.67	907	906
Tomato4-100	Dip	1	63.9	148.66	4	102	62	6.65	6.63	909	907
Tomato1-200	Dip	1	77.23	149.42	4	202	118	6.14	6.1	960	957
Tomato2-200	Dip	1	70.87	145.27	4	197	107	6.2	6.17	957	956
Tomato3-200	Dip	1	65.43	153.69	4	198	92	6.21	6.16	958	956
Tomato4-200	Dip	1	60.75	148.53	4	198	112	6.2	6.17	958	956
Note: Initial solution levels: EW (233 ppm), ORP (834 mV), pH (6.25).											

Table 3C. Chemical analysis of sanitizing solutions for 4-min EW treatment of cabbage (Fig. 3)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			21.3	152.3							
Ctrl2			24.45	157							
Ctrl3			19.87	161.1							
Ctrl4			16.2	160							
Water1	Dip		19.22	178	4			7.02	7	521	520
Water2	Dip		20.34	156.4	4			7.02	7	520	520
Water3	Dip		16.65	161.8	4			7.02	7	522	520
Water4	Dip		14.14	153	4			7.02	7.01	521	520
Cabbage1-50	Dip	1	20.34	157.6	4	49	12	6.76	6.72	825	822
Cabbage2-50	Dip	1	13.49	182	4	46	15	6.75	6.74	825	823
Cabbage3-50	Dip	1	14.86	160	4	48	9	6.75	6.74	825	822
Cabbage4-50	Dip	1	15.6	160	4	44	11	6.76	6.74	825	822
Cabbage1-100	Dip	1	24.74	150.5	4	89	34	6.58	6.56	871	868
Cabbage2-100	Dip	1	20.33	151.7	4	84	29	6.57	6.55	871	868
Cabbage3-100	Dip	1	14.69	146.8	4	86	30	6.59	6.54	871	868
Cabbage4-100	Dip	1	16.78	143.3	4	85	32	6.58	6.56	871	868
Cabbage1-200	Dip	1	23.5	141	4	191	88	6.55	6.52	898	896
Cabbage2-200	Dip	1	21.47	154	4	192	79	6.54	6.53	897	896
Cabbage3-200	Dip	1	26.81	163.5	4	194	101	6.53	6.52	898	895
Cabbage4-200	Dip	1	17.92	155.6	4	181	94	6.53	6.52	898	896
Note: initial solution levels: EW (247 ppm); ORP (852 mV); pH (6.40).											

Table 3D. Chemical analysis of sanitizing solutions for 4-min EW treatment of pepper (Fig. 3)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			27.62	159.61							
Ctrl2			33.85	155.43							
Ctrl3			34.21	142							
Ctrl4			40.15	147.59							
Water1	Dip		35.03	147.03	4			7.12	7.1	478	476
Water2	Dip		37.21	149.25	4			7.12	7.1	477	476
Water3	Dip		40.58	154.65	4			7.13	7.11	478	477
Water4	Dip		44.14	160.44	4			7.13	7.1	477	476
Pepper1-50	Dip	1	47.42	150.8	4	53	32	6.66	6.65	879	878
Pepper2-50	Dip	1	43.25	142.25	4	52	27	6.68	6.66	879	877
Pepper3-50	Dip	1	40.6	147.6	4	48	25	6.65	6.65	879	877
Pepper4-50	Dip	1	37.25	153.6	4	49	27	6.65	6.64	878	875
Pepper1-100	Dip	1	35.27	154.57	4	101	43	6.62	6.6	913	912
Pepper2-100	Dip	1	37.1	150.26	4	96	42	6.62	6.6	910	909
Pepper3-100	Dip	1	39.2	146.65	4	95	50	6.62	6.6	910	909
Pepper4-100	Dip	1	31.53	138.2	4	95	46	6.61	6.59	912	910
Pepper1-200	Dip	1	27.03	131.89	4	201	110	6.48	6.46	926	925
Pepper2-200	Dip	1	31.22	137.62	4	200	105	6.48	6.46	925	924
Pepper3-200	Dip	1	36.71	148.33	4	202	97	6.45	6.45	926	923
Pepper4-200	Dip	1	39.2	158.72	4	196	103	6.46	6.45	926	923
Note: Initial solution levels: EW (229 ppm); ORP (862 mV); pH (6.42)											

Table 3E. Chemical analysis of sanitizing solutions for 4-min EW treatment of spinach (Fig. 3)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			4.84	144							
Ctrl2			5.45	152.5							
Ctrl3			5.89	158							
Ctrl4			6.2	160							
Water1	Dip		9.02	152	4			7.03	6.98	597	596
Water2	Dip		6.77	150	4			7.05	7	597	595
Water3	Dip		5.26	148.5	4			7.03	6.97	596	595
Water4	Dip		4.18	150	4			7.03	6.98	597	595
Spinach1-50	Dip	1	6.32	138.5	4	48	16	6.82	6.8	809	807
Spinach2-50	Dip	1	7.65	140	4	56	18	6.82	6.8	807	805
Spinach3-50	Dip	1	7.01	144	4	52	16	6.82	6.79	808	807
Spinach4-50	Dip	1	6.58	152.5	4	51	14	6.81	6.79	808	807
Spinach1-100	Dip	1	8.72	140	4	98	49	6.76	6.71	814	812
Spinach2-100	Dip	1	6.64	150	4	103	45	6.76	6.72	814	813
Spinach3-100	Dip	1	6.98	150	4	102	40	6.76	6.72	813	812
Spinach4-100	Dip	1	7.04	156.5	4	103	44	6.76	6.71	813	811
Spinach1-200	Dip	1	4.7	150	4	210	110	6.68	6.64	830	828
Spinach2-200	Dip	1	2.68	140	4	205	95	6.68	6.65	829	828
Spinach3-200	Dip	1	3.52	144	4	206	98	6.68	6.64	829	827
Spinach4-200	Dip	1	4.9	144	4	204	86	6.69	6.65	828	827
Note: Initial solution levels: EW (231 ppm); ORP (882 mV); pH (6.25).											

Table 4A. Chemical analysis of sanitizing solutions for 1-min using replenished EW for treatment of grape tomatoes (Fig. 4)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (3 rd end)**	**ORP (start)**	**ORP (3rd end)**
Ctrl1			65.4	143.25							
Ctrl2			60.1	157.6							
Ctrl3			57.14	151.24							
Ctrl4			46.85	150.6							
Water1	Dip		44.1	148.68	1min (20+20+20 sec)			7.08	7.07	552	550
Water2	Dip		48.2	144.3	1min (20+20+20 sec)			7.08	7.07	552	551
Water3	Dip		50.22	150.67	1min (20+20+20 sec)			7.07	7.07	552	551
Water4	Dip		61.73	155.42	1min (20+20+20 sec)			7.08	7.08	550	549
Tomato1-50	Dip	1	76.27	157.82	1min (20+20+20 sec)	48	39	7.14	7.13	852	851
Tomato2-50	Dip	1	85.81	155.25	1min (20+20+20 sec)	51	35	7.15	7.13	852	850
Tomato3-50	Dip	1	74.25	147.62	1min (20+20+20 sec)	46	42	7.15	7.13	852	850
Tomato4-50	Dip	1	69.11	143.7	1min (20+20+20 sec)	46	40	7.16	7.15	853	849
Tomato1-100	Dip	1	60.81	150.44	1min (20+20+20 sec)	98	85	6.8	6.78	927	921
Tomato2-100	Dip	1	56.17	142.37	1min (20+20+20 sec)	92	77	6.75	6.72	927	924
Tomato3-100	Dip	1	54.23	136.6	1min (20+20+20 sec)	95	82	6.75	6.72	927	924
Tomato4-100	Dip	1	58.6	128.52	1min (20+20+20 sec)	95	80	6.75	6.72	928	921
Tomato1-200	Dip	1	49.3	154.37	1min (20+20+20 sec)	198	146	6.38	6.35	963	957
Tomato2-200	Dip	1	62.26	155.25	1min (20+20+20 sec)	195	142	6.38	6.36	965	958
Tomato3-200	Dip	1	54.1	147.62	1min (20+20+20 sec)	194	139	6.35	6.35	968	958
Tomato4-200	Dip	1	52.66	160.22	1min (20+20+20 sec)	195	132	6.36	6.34	965	956
Note: Initial solution levels: EW (213 ppm), ORP (834 mV), pH (6.25).											

Table 4A. Chemical analysis of sanitizing solutions for 2-min using replenished EW for treatment of grape tomatoes (Fig. 4)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (3rd end)**	**ORP (start)**	**ORP (3rd end)**
Ctrl1			58.7	153.14							
Ctrl2			57.23	157.52							
Ctrl3			51.24	146.52							
Ctrl4			60.27	149.8							
Water1	Dip		58.05	155.2	2 min (40+40+40 sec)			7.08	7.07	554	552
Water2	Dip		61.1	163.08	2 min (40+40+40 sec)			7.08	7.06	553	551
Water3	Dip		57.6	147.31	2 min (40+40+40 sec)			7.07	7.06	553	550
Water4	Dip		59.03	140.8	2 min (40+40+40 sec)			7.08	7.07	551	548
Tomato1-50	Dip	1	58.68	145.28	2 min (40+40+40 sec)	48	36	7.12	7.1	854	850
Tomato2-50	Dip	1	77.75	147.2	2 min (40+40+40 sec)	46	38	7.13	7.11	853	850
Tomato3-50	Dip	1	71.3	152.05	2 min (40+40+40 sec)	45	37	7.12	7.1	853	850
Tomato4-50	Dip	1	70.52	158.1	2 min (40+40+40 sec)	46	38	7.08	7.05	853	851
Tomato1-100	Dip	1	74.36	152.25	2 min (40+40+40 sec)	95	82	6.7	6.65	926	924
Tomato2-100	Dip	1	64.65	142.37	2 min (40+40+40 sec)	94	78	6.73	6.7	926	924
Tomato3-100	Dip	1	61.2	128.5	2 min (40+40+40 sec)	96	80	6.72	6.7	925	921
Tomato4-100	Dip	1	53.35	160.22	2 min (40+40+40 sec)	96	74	6.73	6.7	922	917
Tomato1-200	Dip	1	67.34	158.6	2 min (40+40+40 sec)	201	134	6.37	6.35	967	961
Tomato2-200	Dip	1	65.66	150.22	2 min (40+40+40 sec)	203	122	6.35	6.32	967	960
Tomato3-200	Dip	1	54.2	147.53	2 min (40+40+40 sec)	201	135	6.35	6.3	967	959
Tomato4-200	Dip	1	57.17	142.8	2 min (40+40+40 sec)	199	128	6.35	6.3	962	958
Note: Initial solution levels: EW (213 ppm), ORP (834 mV), pH (6.25).											

Table 4A. Chemical analysis of sanitizing solutions for 4-min using replenished EW for treatment of grape tomatoes (Fig. 4)											
Product	**Treatment method**	**EW volume (L)**	**Product (gms)**	**Product (surface area, cm^2)**	**Treatment time (min)**	**PPM Cl- (start)**	**PPM Cl- (finish)**	**pH (start)**	**pH (3rd end)**	**ORP (start)**	**ORP (3rd end)**
Ctrl1			48.61	154.3							
Ctrl2			55.2	158.56							
Ctrl3			51.21	146.5							
Ctrl4			59.78	149.82							
Water1	Dip		53.22	146.25	4 min (80+80+80 sec)			7.06	7.05	553	551
Water2	Dip		56.67	148.6	4 min (80+80+80 sec)			7.05	7.02	552	549
Water3	Dip		58.21	139.1	4 min (80+80+80 sec)			7.05	7.02	550	547
Water4	Dip		48.9	149.56	4 min (80+80+80 sec)			7.06	7.03	550	547
Tomato1-50	Dip	1	82.65	154.2	4 min (80+80+80 sec)	49	19	7.1	7.05	858	851
Tomato2-50	Dip	1	58.19	146.65	4 min (80+80+80 sec)	52	26	7.1	7.06	857	848
Tomato3-50	Dip	1	74.33	143.27	4 min (80+80+80 sec)	52	27	7.11	7.04	856	845
Tomato4-50	Dip	1	75.1	150.08	4 min (80+80+80 sec)	51	22	7.09	7.02	856	846
Tomato1-100	Dip	1	74.36	158.1	4 min (80+80+80 sec)	95	71	6.69	6.6	928	923
Tomato2-100	Dip	1	64.65	159.21	4 min (80+80+80 sec)	101	68	6.68	6.58	928	921
Tomato3-100	Dip	1	59.21	158.6	4 min (80+80+80 sec)	100	65	6.68	6.56	926	920
Tomato4-100	Dip	1	52	134.8	4 min (80+80+80 sec)	100	67	6.65	6.59	928	921
Tomato1-200	Dip	1	45.56	160.3	4 min (80+80+80 sec)	196	116	6.32	6.25	971	957
Tomato2-200	Dip	1	60.58	158.71	4 min (80+80+80 sec)	195	118	6.28	6.2	971	957
Tomato3-200	Dip	1	53.21	150.22	4 min (80+80+80 sec)	198	109	6.27	6.2	970	958
Tomato4-200	Dip	1	57.8	143.67	4 min (80+80+80 sec)	196	98	6.27	6.18	970	954
Note: Initial solution levels: EW (213 ppm), ORP (834 mV), pH (6.25).											

Table 5A. Chemical analysis of sanitizing solutions for 1-min LA treatment of grape tomatoes (Fig. 5)									
Products	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(1min)			142.579	58.7					
Ctrl2(1min)			152.337	62.5					
Ctrl3(1min)			149.62	65.1					
Ctrl4(1min)			156.22	61.23					
Water 1	Dip		145.26	54.22	1	6.74	6.73	472	472
Water 2	Dip		159.82	51.36	1	6.74	6.73	473	472
Water 3	Dip		160.21	54.29	1	6.75	6.73	473	472
Water 4	Dip		151.4	48.1	1	6.74	6.73	473	471
1%									
Tomato1(1min)	Dip	1	152.186	79	1	2.39	2.4	285	288
Tomato2(1min)	Dip	1	148.5	81.2	1	2.38	2.39	285	286
Tomato3(1min)	Dip	1	155.61	80.6	1	2.39	2.39	286	288
Tomato4(1min)	Dip	1	154.273	82.3	1	2.39	2.39	285	286
2%									
Tomato1(1min)	Dip	1	153.437	89.5	1	2.1	2.12	341	343
Tomato2(1min)	Dip	1	156.22	84.2	1	2.12	2.13	341	342
Tomato3(1min)	Dip	1	151.653	79.7	1	2.1	2.12	340	342
Tomato4(1min)	Dip	1	147.265	77.3	1	2.11	2.13	341	343
4%									
Tomato1(1min)	Dip	1	144.294	93	1	1.72	1.8	416	419
Tomato2(1min)	Dip	1	148.254	86.6	1	1.7	1.8	416	418
Tomato3(1min)	Dip	1	150.28	90.8	1	1.7	1.75	416	416
Tomato4(1min)	Dip	1	152.645	72.7	1	1.71	1.8	416	417
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 5A. Chemical analysis of sanitizing solutions for 2-min LA treatment of grape tomatoes (Fig. 5)									
Product	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(2min)			153.26	60.1					
Ctrl2(2min)			150.65	57.2					
Ctrl3(2min)			148.27	54.11					
Ctrl4(2min)			149.62	56.72					
Water 1	Dip		154.24	53.2	2	6.72	6.71	471	470
Water 2	Dip		152.2	50.19	2	6.72	6.72	468	465
Water 3	Dip		148.7	46.8	2	6.72	6.71	471	470
Water 4	Dip		146.25	48.22	2	6.71	6.7	471	469
1%									
Tomato1(2min)	Dip	1	150.30	71	2	2.39	2.4	285	287
Tomato2(2min)	Dip	1	149.26	70.6	2	2.39	2.41	286	288
Tomato3(2min)	Dip	1	152.45	81.6	2	2.39	2.4	285	289
Tomato4(2min)	Dip	1	150.82	84.2	2	2.38	2.4	285	289
2%									
Tomato1(2min)	Dip	1	151.55	60	2	2.1	2.3	341	344
Tomato2(2min)	Dip	1	154.25	66.7	2	2.1	2.2	341	345
Tomato3(2min)	Dip	1	149.25	77.9	2	2.11	2.2	340	344
Tomato4(2min)	Dip	1	153.37	80.1	2	2.1	2.2	341	346
4%									
Tomato1(2min)	Dip	1	153.43	95	2	1.7	1.85	416	420
Tomato2(2min)	Dip	1	156.88	71.9	2	1.71	1.84	416	420
Tomato3(2min)	Dip	1	149.72	76.6	2	1.71	1.86	416	419
Tomato4(2min)	Dip	1	150.25	80.4	2	1.7	1.85	418	420
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 5A. Chemical analysis of sanitizing solutions for 4-min LA treatment of grape tomatoes (Fig. 5)									
Product	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(4min)			145.67	49.8					
Ctrl2(4min)			153.28	55.2					
Ctrl3(4min)			150.26	45.2					
Ctrl4(4min)			147.26	51.23					
Water 1	Dip		146.2	45.97	4	6.76	6.75	478	476
Water 2	Dip		148.55	46.58	4	6.75	6.74	479	474
Water 3	Dip		149.37	45.1	4	6.75	6.74	480	478
Water 4	Dip		156.02	52.7	4	6.76	6.75	480	478
1%									
Tomato1(4min)	Dip	1	150.30	58.5	4	2.39	2.42	285	288
Tomato2(4min)	Dip	1	144.48	62.7	4	2.39	2.42	285	289
Tomato3(4min)	Dip	1	148.28	71.2	4	2.39	2.42	285	289
Tomato4(4min)	Dip	1	155.41	74.4	4	2.39	2.43	285	290
2%									
Tomato1(4min)	Dip	1	144.29	92.5	4	2.1	2.3	341	347
Tomato2(4min)	Dip	1	146.25	88.1	4	2.1	2.4	340	347
Tomato3(4min)	Dip	1	150.66	79.3	4	2.1	2.3	341	348
Tomato4(4min)	Dip	1	152.54	77.4	4	2.2	2.4	341	349
4%									
Tomato1(4min)	Dip	1	150.30	52.5	4	1.7	1.88	417	424
Tomato2(4min)	Dip	1	152.35	69.1	4	1.72	1.89	417	423
Tomato3(4min)	Dip	1	158.90	70.2	4	1.72	1.85	417	426
Tomato4(4min)	Dip	1	156.71	56.4	4	1.7	1.91	416	424
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 6A. Chemical analysis of sanitizing solutions for 1-min LA treatment of baby carrots (Fig. 6)									
Product	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(1min)			154.25	49.2					
Ctrl2(1min)			148.37	54.3					
Ctrl3(1min)			146.25	51.2					
Ctrl4(1min)			144.13	56.4					
Water 1	Dip		150.27	51.6	1	6.74	6.74	474	473
Water 2	Dip		144.2	54.9	1	6.74	6.74	474	473
Water 3	Dip		143.68	57.23	1	6.74	6.75	474	473
Water 4	Dip		140.9	52.06	1	6.75	6.74	473	470
1%									
Carrot1(1min)	Dip	1	152.41	86.6	1	2.39	2.6	285	291
Carrot2(1min)	Dip	1	150.65	88.2	1	2.38	2.61	285	292
Carrot3(1min)	Dip	1	154.22	77.6	1	2.39	2.6	285	290
Carrot4(1min)	Dip	1	148.65	72.5	1	2.39	2.64	285	290
2%									
Carrot1(1min)	Dip	1	147.95	72	1	2.1	2.3	340	351
Carrot2(1min)	Dip	1	150.86	68.1	1	2.1	2.32	341	350
Carrot3(1min)	Dip	1	147.25	58.3	1	2.1	2.32	340	350
Carrot4(1min)	Dip	1	150.86	71.3	1	2.1	2.35	340	349
4%									
Carrot1(1min)	Dip	1	152.41	84.5	1	1.7	1.9	416	421
Carrot2(1min)	Dip	1	154.22	78.1	1	1.72	1.95	416	422
Carrot3(1min)	Dip	1	148.65	72.4	1	1.7	1.94	416	421
Carrot4(1min)	Dip	1	152.41	80.3	1	1.7	1.94	416	421
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 6A. Chemical analysis of sanitizing solutions for 2-min LA treatment of baby carrots (Fig. 6)									
Product	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(2min)			156.25	49.7					
Ctrl2(2min)			150.44	54.2					
Ctrl3(2min)			148.7	49.82					
Ctrl4(2min)			147.25	46.25					
Water 1			146.25	49.28	2	6.75	6.74	472	471
Water 2			144.82	57.1	2	6.76	6.75	472	470
Water 3			143.69	54.6	2	6.76	6.75	473	471
Water 4			147.26	54.92	2	6.74	6.72	473	471
1%									
Carrot1(2min)	Dip	1	151.55	62.5	2	2.39	2.65	285	298
Carrot2(2min)	Dip	1	147.25	55.4	2	2.4	2.65	285	298
Carrot3(2min)	Dip	1	144.36	58.1	2	2.39	2.64	285	230
Carrot4(2min)	Dip	1	152.58	52.8	2	2.39	2.67	286	231
2%									
Carrot1(2min)	Dip	1	162.33	87.5	2	2.12	2.35	340	356
Carrot2(2min)	Dip	1	151.55	59.8	2	2.1	2.35	340	361
Carrot3(2min)	Dip	1	152.43	63.2	2	2.11	2.31	340	361
Carrot4(2min)	Dip	1	152.41	72.3	2	2.11	2.34	341	360
4%									
Carrot1(2min)	Dip	1	142.04	56.5	2	1.71	1.9	418	435
Carrot2(2min)	Dip	1	144.39	61.3	2	1.71	1.89	418	432
Carrot3(2min)	Dip	1	150.63	72.5	2	1.7	1.89	418	435
Carrot4(2min)	Dip	1	147.95	64.2	2	1.7	1.92	417	432
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 6A. Chemical analysis of sanitizing solutions for 4-min LA treatment of baby carrots (Fig. 6)									
Product	**Treatment method**	**Lactic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**Treatment time (min)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1(4min)			154.36	58.2					
Ctrl2(4min)			152.65	64.4					
Ctrl3(4min)			149.82	59.15					
Ctrl4(4min)			147.2	46.8					
Water 1	Dip		147.12	54.1	4	6.72	6.72	473	472
Water 2	Dip		141.23	51.2	4	6.72	6.73	474	473
Water 3	Dip		149.8	56.7	4	6.75	6.74	473	472
Water 4	Dip		146.57	49.77	4	6.74	6.73	474	470
1%									
Carrot1(4min)	Dip	1	151.55	70	4	2.39	2.72	286	311
Carrot2(4min)	Dip	1	154.22	75.3	4	2.38	2.73	285	315
Carrot3(4min)	Dip	1	148.65	72.6	4	2.38	2.73	285	311
Carrot4(4min)	Dip	1	144.33	59.7	4	2.39	2.75	286	312
2%									
Carrot1(4min)	Dip	1	157.82	65.5	4	2.12	2.41	340	368
Carrot2(4min)	Dip	1	155.34	68.2	4	2.12	2.42	340	370
Carrot3(4min)	Dip	1	152.65	54.2	4	2.1	2.4	341	371
Carrot4(4min)	Dip	1	146.37	71.9	4	2.11	2.43	340	371
4%									
Carrot1(4min)	Dip	1	156.64	72	4	1.71	2.01	416	456
Carrot2(4min)	Dip	1	158.25	71.5	4	1.73	2.01	416	462
Carrot3(4min)	Dip	1	154.22	67.4	4	1.72	2.02	417	462
Carrot4(4min)	Dip	1	146.37	57.6	4	1.7	2	416	464
Note: Initial solution levels: LA (1%), ORP (286 mV), pH (2.38); LA (2%), ORP (340 mV), pH (2.09); LA (4%), ORP (415 mV), pH (1.7)									

Table 7A. Chemical analysis of sanitizing solutions for PAA treatment of grape tomatoes (Fig. 7)								
Products	**Treatment method**	**Peroxyacetic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			154.2	49.28				
Ctrl2			150.29	46.64				
Ctrl3			156.74	49.27				
Ctrl4			148.07	51.68				
Water 1	Dip		146.28	51.6	6.75	6.74	467	465
Water 2	Dip		149.57	54.9	6.75	6.74	467	466
Water 3	Dip		151.2	57.21	6.76	6.74	467	465
Water 4	Dip		150.46	60.12	6.76	6.73	467	466
1 min								
Tomato1	Dip	1	147.26	75.2	3.27	3.25	472	471
Tomato2	Dip	1	144.21	74.6	3.27	3.25	473	471
Tomato3	Dip	1	146.8	79.1	3.27	3.26	475	473
Tomato4	Dip	1	156.24	68.09	3.26	3.24	473	473
2 min								
Tomato1	Dip	1	164.2	74.1	3.26	3.23	474	473
Tomato2	Dip	1	157.26	76.82	3.25	3.23	473	472
Tomato3	Dip	1	159.4	59.4	3.26	3.25	473	472
Tomato4	Dip	1	143.82	70.12	3.26	3.24	473	473
4 min								
Tomato1	Dip	1	154.2	74.1	3.26	3.26	473	471
Tomato2	Dip	1	153.24	49.68	3.26	3.25	473	470
Tomato3	Dip	1	150.49	59.28	3.27	3.24	472	470
Tomato4	Dip	1	147.68	54.6	3.28	3.24	471	470
Note: Initial solution levels: PAA (50 ppm), ORP (473 mV), pH (3.26).								

Table 7A. Chemical analysis of sanitizing solutions for PAA treatment of baby carrots (Fig. 7)								
Products	**Treatment method**	**Peroxyacetic acid volume (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			152.92	34.57				
Ctrl2			153.13	38.91				
Ctrl3			156.28	46.15				
Ctrl4			144.15	48.91				
Water 1	Dip		156.23	46.2	6.74	6.72	471	469
Water 2	Dip		154.1	37.8	6.76	6.74	471	469
Water 3	Dip		150.2	46.26	6.74	6.72	468	467
Water 4	Dip		149.8	46.5	6.74	6.73	468	466
1 min								
Carrot1	Dip	1	152.18	49.27	3.29	3.3	481	478
Carrot2	Dip	1	146.59	46.82	3.29	3.29	481	478
Carrot3	Dip	1	148.27	45.56	3.29	3.29	480	474
Carrot4	Dip	1	146.05	49.06	3.29	3.3	481	478
2 min								
Carrot1	Dip	1	159.28	46.8	3.29	3.31	479	479
Carrot2	Dip	1	154.77	45.27	3.28	3.3	478	478
Carrot3	Dip	1	150.42	49.16	3.28	3.29	481	481
Carrot4	Dip	1	160.2	40.2	3.27	3.29	482	480
4 min								
Carrot1	Dip	1	154.23	51.7	3.27	3.3	482	482
Carrot2	Dip	1	151.27	54.09	3.27	3.3	485	485
Carrot3	Dip	1	147.62	45.67	3.28	3.3	485	486
Carrot4	Dip	1	145.07	49.82	3.31	3.32	486	485
Note: Initial solution levels: PAA (50 ppm), ORP (481 mV), pH (3.29).								

Table 8A. Chemical analysis of sanitizing solutions for LA (0.5%) + EW (25 ppm) treatment of grape tomatoes (Fig. 8)								
Product	**Treatment method**	**Lactic acid (1% 500ml)+ E.W.(50ppm 500ml) (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			148.79	55.16				
Ctrl2			150.23	57.24				
Ctrl3			152.26	49.88				
Ctrl4			149.82	56.02				
Water 1	Dip		154.26	54.01	6.75	6.73	469	467
Water 2	Dip		151.22	53.29	6.75	6.72	469	469
Water 3	Dip		147.62	46.87	6.75	6.73	468	467
Water 4	Dip		144.31	44.1	6.75	6.72	469	468
1min								
Tomato1	Dip	1	151.56	53.45	2.5	2.52	674	674
Tomato2	Dip	1	148.18	48.15	2.5	2.52	674	675
Tomato3	Dip	1	151.56	55.58	2.51	2.52	674	675
Tomato4	Dip	1	154.24	52.26	2.5	2.53	676	676
2min								
Tomato1	Dip	1	140.91	41.04	2.52	2.54	674	675
Tomato2	Dip	1	144.80	41.81	2.5	2.54	674	676
Tomato3	Dip	1	150.31	74.84	2.5	2.54	674	676
Tomato4	Dip	1	152.22	66.25	2.51	2.54	674	677
4min								
Tomato1	Dip	1	152.19	73.23	2.5	2.54	674	676
Tomato2	Dip	1	144.29	56.52	2.5	2.55	674	678
Tomato3	Dip	1	154.37	81.01	2.5	2.55	674	678
Tomato4	Dip	1	151.56	77.25	2.5	2.57	675	680
Note: Initial solution levels: LA (0.5%) + EW (25 ppm) (206 ppm), ORP (805 mV), pH (6.07).								

Table 8A. Chemical analysis of sanitizing solutions for LA (0.5%) + EW (25 ppm) treatment of baby carrots (Fig. 8)								
Product	**Treatment method**	**Lactic acid (1% 500ml)+ E.W.(50ppm 500ml) (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			155.60	80.41				
Ctrl2			150.43	74.12				
Ctrl3			148.69	65.8				
Ctrl4			150.24	65.27				
Water 1	Dip		146.28	76.25	6.78	6.76	470	468
Water 2	Dip		145.26	74.56	6.78	6.78	470	469
Water 3	Dip		149.67	64.09	6.76	6.75	470	469
Water 4	Dip		156.80	59.88	6.78	6.76	469	468
1min								
Carrots1	Dip	1	184.62	73.01	2.5	2.51	674	674
Carrots2	Dip	1	151.56	64.92	2.5	2.5	674	675
Carrots3	Dip	1	144.30	72.28	2.5	2.51	674	675
Carrots4	Dip	1	148.57	68.65	2.5	2.52	674	675
2min								
Carrots1	Dip	1	164.87	101.83	2.51	2.52	674	675
Carrots2	Dip	1	136.41	60.3	2.5	2.51	675	675
Carrots3	Dip	1	148.80	112.27	2.51	2.51	674	675
Carrots4	Dip	1	152.22	84.75	2.5	2.53	674	676
4min								
Carrots1	Dip	1	169.10	84.82	2.5	2.53	675	676
Carrots2	Dip	1	147.90	70.44	2.51	2.53	675	676
Carrots3	Dip	1	138.80	93.66	2.5	2.52	674	675
Carrots4	Dip	1	145.59	90.25	2.5	2.53	674	675
Note: Initial solution levels: LA (0.5%) + EW (25 ppm) (206 ppm), ORP (805 mV), pH (6.07).								

Table 9A. Chemical analysis of sanitizing solutions for LA (1 %) + EW (50 ppm) treatment of grape tomatoes (Fig. 9)								
Product	**Treatment method**	**Lactic acid (2% 500ml)+ EW (100ppm 500ml) (L)**	**Product (surface area, cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			158.26	64.2				
Ctrl2			154.33	63.59				
Ctrl3			147.10	58.1				
Ctrl4			149.25	54.71				
Water 1	Dip		150.26	53.46	6.74	6.72	471	470
Water 2	Dip		154.23	59.8	6.73	6.7	472	471
Water 3	Dip		155.80	61.28	6.7	6.7	471	470
Water 4	Dip		147.61	54.81	6.71	6.7	471	470
1min								
Tomato1	Dip	1	146.80	59.8	3.67	3.65	635	635
Tomato2	Dip	1	144.59	65.7	3.65	3.62	635	635
Tomato3	Dip	1	143.70	62.24	3.67	3.65	635	633
Tomato4	Dip	1	138.60	60.19	3.65	3.63	637	636
2min								
Tomato1	Dip	1	159.80	46.18	3.65	3.64	635	632
Tomato2	Dip	1	156.52	51.8	3.63	3.62	634	633
Tomato3	Dip	1	142.20	54.27	3.64	6.61	634	633
Tomato4	Dip	1	134.19	56.98	3.64	6.61	634	636
4min								
Tomato1	Dip	1	149.87	54.1	3.65	3.62	632	631
Tomato2	Dip	1	146.52	51.29	3.61	3.6	632	632
Tomato3	Dip	1	142.10	46.8	3.61	3.6	635	636
Tomato4	Dip	1	158.70	44.52	3.63	3.61	632	632
Note: Initial solution levels: LA (1 %) + EW (50 ppm) (219 ppm), ORP (812 mV), pH (6.18).								

Table 9A. Chemical analysis of sanitizing solutions for LA (1 %) + EW (50 ppm) treatment of baby carrots (Fig. 9)								
Product	**Treatment method**	**Lactic acid (1% 500ml)+ E.W.(50ppm 500ml) (L)**	**Product (surface area,cm^2)**	**Product (gms)**	**pH (start)**	**pH (end)**	**ORP (start)**	**ORP (end)**
Ctrl1			151.35	45.2				
Ctrl2			154.20	49.82				
Ctrl3			146.70	46.7				
Ctrl4			138.94	48.11				
Water 1	Dip		154.21	51.24	6.75	6.75	472	470
Water 2	Dip		159.80	47.62	6.75	6.73	472	471
Water 3	Dip		161.70	49.82	6.76	6.75	471	470
Water 4	Dip		121.98	56.27	6.75	6.75	472	470
1min								
Carrots1	Dip	1	167.50	60.1	2.48	2.5	670	669
Carrots2	Dip	1	154.60	62.45	2.51	2.5	670	670
Carrots3	Dip	1	150.24	46.75	2.53	2.48	671	670
Carrots4	Dip	1	150.24	47.18	2.51	2.5	671	670
2min								
Carrots1	Dip	1	156.70	44.21	2.53	2.5	672	671
Carrots2	Dip	1	149.50	49.87	2.53	2.51	671	671
Carrots3	Dip	1	146.82	46.5	2.54	2.51	671	671
Carrots4	Dip	1	137.54	51.2	2.53	2.51	671	671
4min								
Carrots1	Dip	1	149.86	51.6	2.52	2.48	676	675
Carrots2	Dip	1	144.21	54.76	2.5	2.51	676	675
Carrots3	Dip	1	138.75	59.8	2.52	2.49	678	675
Carrots4	Dip	1	156.24	37.29	2.5	2.49	676	674
Note: Initial solution levels: LA (1 %) + EW (50 ppm) (219 ppm), ORP (812 mV), pH (6.18).								

VITA

Jiameng Wang

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF HYPOCHLOROUS ACID (ELECTROLYZED WATER), LACTIC ACID, AND PEROXYACETIC ACID AS SANITIZERS FOR FRESH VEGETABLES

Major Field: Food Science

Biographical:

Personal Data: Born in Beijing, People's Republic of China on July 17, 1984, the daughter of Bin Wang and Lu Lu.

Education: Graduated from Beijing No. 11 middle School in Beijing, China in July, 2002; Graduated from Beijing Agricultural University in Beijing, China in July, 2006; Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

Experience: Employed in Beijing Cadbury Company from September 2006 till August 2007 in Beijing, China; Employed as Dr. Peter Muriana's graduate research assistant for Oklahoma State University from August 2008 till May 2011.

Professional Memberships: Institute of Food Technologists.

Name: Jiameng Wang Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF HYPOCHLOROUS ACID (ELECTROLYZED WATER), LACTIC ACID, AND PEROXYACETIC ACID AS SANITIZERS FOR FRESH VEGETABLES

Pages in Study: 104 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: Our objectives were to determine the effectiveness of hypochlorous acid (i.e., electrolyzed water), lactic acid, peroxyacetic acid, and sodium thiocyanate against indigenous bacteria on a standardized surface treatment area (150 cm^2) of different types of vegetables, for their potential application as antimicrobial treatments for fresh produce. Vegetable samples were dipped in three concentrations of electrolyzed water (50, 100, or 200 ppm Cl^-), lactic acid (1%, 2%, or 4%), and peroxyacetic acid (50 ppm) to control microbial populations at three dwell treatment times (1, 2, or 4 min). The effects of electrolyzed water (25 or 50 ppm) combined with lactic acid (0.5% or 1%), and electrolyzed water dip followed by sodium thiocyanate spray treatment were also tested. Samples were drained and rinsed with 50 ml buffer peptone water (BPW), and stomached to resuspend remaining viable cells. Serial dilutions were made in 0.1% BPW and plated on Plated Count Agar (PCA).

Findings and Conclusions: Our results suggest that, a standardized surface area of produce will better reflect the relative effectiveness of different antimicrobials on various types of produce than evaluation based on weight. All dipping solutions tested in our study were capable of reducing microbial populations to some extent, but, the antimicrobial effects were dependent on concentrations, treatment times, and types of produce. Electrolyzed water (50 ppm), with a 1 min dipping treatment time, may be effective in reducing microbial loads from some food produce surfaces whereas other types of produce may require higher levels and/or longer treatment times. Lactic acid was tested in combination with electrolyzed water and performed well in reducing bacteria on food produce surfaces at lower levels of LA or EW than if either were used alone. The bactericidal activity of electrolyzed water is more pronounced on firm “skin” (grape tomatoes) than rough “skin” (baby carrots) vegetables.

ADVISER’S APPROVAL: PETER M. MURIANA